Plant Physiol. (1985) 79, 1–6
0032-0889/85/79/0001/06/$01.00/0

Chloroplast Biogenesis 49[1]

DIFFERENCES AMONG ANGIOSPERMS IN THE BIOSYNTHESIS AND ACCUMULATION OF MONOVINYL AND DIVINYL PROTOCHLOROPHYLLIDE DURING PHOTOPERIODIC GREENING

Received for publication January 24, 1985 and in revised form April 30, 1985

EDWARD E. CAREY[2] AND CONSTANTIN A. REBEIZ*
Laboratory of Plant Pigment Biochemistry and Photobiology, Department of Horticulture, University of Illinois, Urbana, Illinois 61801

ABSTRACT

Various angiosperms differed in their monovinyl and divinyl protochlorophyllide biosynthetic capabilities during the dark and light phases of photoperiodic growth. Some plant species such as *Cucumis sativus* L., *Brassica juncea* (L.) Coss., *Brassica kaber* (DC.) Wheeler, and *Portulaca oleracea* L. accumulated mainly divinyl protochlorophyllide at night. Monocotyledonous species such as *Avena sativa* L., *Hordeum vulgare* L., *Triticum secale* L., *Zea mays* L., and some dicotyledonous species such as *Phaseolus vulgaris* L., *Glycine max* (L.) Merr., *Chenopodium album* L., and *Lycopersicon esculentum* L. accumulated mainly monovinyl protochlorophyllide at night.

Under low light intensities meant to simulate the first 60 to 80 minutes following daybreak divinyl protochlorophyllide appeared to contribute much more to chlorophyll formation than monovinyl protochlorophyllide in species such as *Cucumis sativus* L. Under the same light conditions, species which accumulated mainly monovinyl protochlorophyllide at night appeared to form chlorophyll preferably via monovinyl protochlorophyllide.

These results were interpreted in terms of: (a) a differential contribution of monovinyl and divinyl protochlorophyllide to chlorophyll formation at daybreak in various plant species; and (b) a differential regulation of the monovinyl and divinyl protochlorophyllide biosynthetic routes by light and darkness.

Protochlorophyllide is the precursor of most of the Chl *a* in greening etiolated plants (26), and in plants growing under natural photoperiods (7, 8). In photoperiodically grown plants, the Pchlide[3] which accumulates at night is photoconverted at daybreak to Chlide *a* (8), which is then converted to Chl *a*.

The Pchlide pool of higher plants was recently shown to consist of both MV[4] and DV components which appear to contribute independently to the formation of Chl *a*. The evidence for this is as follows: (a) MV Pchlide is photoconverted to MV Chlide *a*, and DV Pchlide is photoconverted to DV Chlide *a* (4, 5, 9, 20, 27); this observation was recently confirmed by others (11); (b) the nascent MV Chlide *a* is converted to MV Chl *a* (4) and the bulk of the nascent DV Chlide *a* is converted first to MV Chlide *a* (10) and then to MV Chl *a* (23); (c) a small fraction of the DV Chlide *a* is also converted to DV Chl *a* (21).

The proportion of MV and DV Pchlide in etiolated and greening plants has been shown to be influenced by light and to vary among species (2, 3, 5, 23). For example, etiolated maize, barley, and bean seedlings accumulate mostly or entirely MV Pchlide, while etiolated cucumber cotyledons accumulate a mixture of MV and DV Pchlide (2, 3). Following a series of light-dark treatments, etiolated cucumber and bean seedlings form only DV Pchlide, barley forms MV Pchlide, and maize forms a mixture of MV and DV Pchlide (5). Finally both photoperiodically grown and etiolated cucumber seedlings accumulate mainly DV Pchlide under illumination (3, 23).

The effect of alternating light and darkness on the MV and DV Pchlide biosynthetic capabilities of plants growing under natural photoperiods has not been investigated. Nor is it known to what extent differences in the MV and DV Pchlide biosynthetic capabilities contribute to the Chl biosynthetic heterogeneity in various plant species. We have therefore undertaken a systematic research effort aimed at assessing the occurrence and extent of this Chl biosynthetic heterogeneity in green plants. In this work, we report that various angiosperms growing under photoperiodic conditions differ drastically in their MV and DV Pchlide biosynthetic capabilities, both in darkness (*i.e.* at night) and in the light.

MATERIALS AND METHODS

Plant Material and Growth Conditions. The crop plant species used in this study were barley (*Hordeum vulgare* L. cv Kentucky No. 1), bean (*Phaseolus vulgaris* L. cv Red Kidney), cotton (*Gossypium hirsutum* l. cv Coker 315), cucumber (*Cucumis sativus* L. cv Beit Alpha MR), maize (*Zea mays* L. cv Funks G4646), oat (*Avena sativa* L. cv Centennial), soybean (*Glycine max* [L.] Merr. cv Williams 82), tomato (*Lycopersicon esculentum* L. cv Jet Star), and wheat (*Triticum secale* L. cv Auburn). The weed species used were lambsquarters (*Chenopodium album* L.), mustard (a mixture of *Brassica juncea* [L.] Coss. and *B. kaber* [DC.] Wheeler.), pigweed (*Amaranthus retroflexus* L.), and common purslane (*Portulaca oleracea* L.).

Seed of all plant species, except purslane, was germinated and grown in moist vermiculite in glass containers (7 cm deep × 9 cm in diameter) in a growth chamber. Twenty-d-old greenhouse grown purslane plants were transferred to the growth chamber 1 week prior to use. Seedlings were grown under mixed cool-white fluorescent and incandescent lights under a photoperiod of 14 h light/10 h dark. The light intensity was about 900 ft-c and the

[1] Supported by Research grant PCM 83-07660 from the National Science Foundation, by funds from the Illinois Agricultural Experiment Station (C.A.R.), and by a Graduate Research Assistantship (E.E.C.).

[2] Present address: Cassava Improvement Program, C.I.A.T., Apartado Aereo 6713, Cali, Colombia.

[3] Unless preceded by MV or DV, the terms Pchlide, Chlide, and Chl are used generically to designate metabolic pools that may consist of MV and DV components.

[4] Abbreviations: MV, monovinyl; DV, divinyl; ALA: δ-aminolevulinic acid; dicot, dicotyledonous; monocot, monocotyledonous.

temperature was maintained at 28°C during the day and at 20°C at night. Seedlings were grown for the lengths of time required to provide suitable quantities of plant material for analysis.

Light and Dark Treatments. The type of Pchlide that was likely to contribute to Chl biosynthesis immediately following daybreak was determined by analysis of the quantities and rate of change of the MV and DV Pchlides that accumulated during the dark phase of a particular photoperiod. Likewise, the type of Pchlide that contributed to greening during the first 60 to 80 min following daybreak was determined by analysis of the amounts and rate of change of MV and DV Pchlide formation under 100 ft-c of white fluorescent light, beginning with the onset of the light phase of a particular photoperiod. The low light intensity used in these studies was meant to simulate the low insolation experienced by plants at daybreak. Because of the broad electronic spectral absorption properties of Pchlide holochromes and their photoconvertibility by either blue or red light (14), the use of white fluorescent light instead of a red-enriched light source was not likely to make a difference in the observed results. To monitor changes in Pchlide content, during the dark phase of the photoperiod, the plants were wrapped in aluminum foil at the end of 14 h of illumination and were placed in a dark room for various lengths of time prior to extraction. Extraction was carried out under a green safelight. To monitor changes in Pchlide content during the light phase of the photoperiod, wrapped plants were removed from the darkroom at the end of 10 h of darkness, unwrapped, and placed in a water bath at 28°C under 100 ft-c of cool-white fluorescent light for the desired length of time. The low light intensity was meant to approximate the average insolation following daybreak. At the end of the light treatment, the plants were harvested and extracted under subdued laboratory light.

Extraction of Pchlide. Fresh tissue was hand homogenized by mortar and pestle in enough acetone:0.1 N HN_4OH (9:1 v/v) to give a final ratio of 3 g tissue to 20 ml of extraction medium. The homogenate was centrifuged at 39,000g for 10 min and the supernatant containing the extracted pigments was collected by decantation. The Pchlide and other monoesterified pigments were separated from the fully esterified pigments by extraction of the acetone extract with an equal volume of hexane followed by an additional extraction with one-third volume of hexane. The Pchlide remained in the hexane extracted acetone fraction. An aliquot of this solution was used for quantitative pigment determination at room temperature. Pchlide was next transferred to ether by addition to the hexane-extracted acetone solution of one-seventieth of its volume of 0.5 M KH_2PO_4 (pH 7.0) and one-seventeenth of its volume of saturated NaCl, followed by a few ml of peroxide-free diethyl ether. The Pchlide in ether was further purified by addition of an equal volume of acetone:ether (1:1 v/v) followed by mixing with a 5-volume excess of 0.37 M KH_2PO_4 (pH 7.0). Following the addition of a few ml of ether, the ether epiphase was collected and used for 77 K spectrofluorometric analysis.

Spectrofluorometry. Fluorescence spectra were recorded on a fully corrected, photon counting spectrofluorometer model SLM 8000 DS, equipped with two red-sensitive extended S20 photomultipliers (EMI 9658) and interfaced with a Hewlett-Packard microcomputer model 9825A. Pigment solutions were monitored either at room temperature in a cylindrical microcell 3 mm in diameter or at 77 K as described previously (8). At room temperature, excitation and emission bandwidths of 2 nm were used. At 77 K, the emission bandwidth was varied from 0.5 to 4 nm depending on signal intensity. The photon count was integrated for 0.5 s at each 1-nm increment.

Quantitation of MV and DV Pchlide. The amount of MV and DV Pchlide in a sample was determined from the total content of Pchlide and from the ratio of MV to DV Pchlide in the sample. The total amount of Pchlide was determined by spectrofluorometry at room temperature as described elsewhere (19, 22). The ratio of MV to DV Pchlide was determined from fluorescence excitation spectra recorded at 77 K in ether, using the following equations:

$$\text{MV Pchlide (E437F625)} = 1.093\ \text{(E437F625)} - 0.624\ \text{(E451F625)} - 0.217\ \text{(E424F625)} \quad (1)$$

$$\text{DV Pchlide (E451F625)} = 1.070\ \text{(E451F625)} - 0.020\ \text{(E437F625)} - 0.163\ \text{(E424F625)} \quad (2)$$

where: MV Pchlide (E437F625) is the deconvoluted net Soret excitation amplitude at 437 nm of the MV Pchlide component of the MV plus DV Pchlide pool; DV Pchlide (E451F625) is the deconvoluted net Soret excitation amplitude at 451 nm of the DV Pchlide component of the MV plus DV Pchlide pool; (E437F625) is the Soret excitation amplitude at 437 nm of the MV plus DV Pchlide mixture which is recorded at the emission maximum of Pchlide (625 nm); (E451F625) is the Soret excitation amplitude at 451 nm of the MV plus DV Pchlide mixture which is recorded at the emission maximum of Pchlide (625 nm); and (E424F625) is the Soret excitation amplitude at 424 nm of the MV plus DV Pchlide mixture which is recorded at the emission maximum of Pchlide (625 nm), etc.

The ratio of the net Soret excitation amplitudes calculated from Eq. 1 and 2 were converted to relative concentration ratios by reference to a standard calibration curve which plotted net fluorescence amplitude ratios against MV/DV Pchlide concentration ratios. the derivation of Eq. 1 and 2 and the development of the calibration curve have been described elsewhere (25).

Protein Determination. The acetone-insoluble residue which was left after centrifugation of the tissue homogenate was resuspended in distilled H_2O with an all glass tissue grinder. Total proteins were determined by the biuret method on an aliquot of the suspension after delipidation (18).

Spectrophotometry. Absorption spectra were recorded with an Aminco dual-wavelength spectrophotometer model DW-2 operated in the split beam mode at a slit width of 2 nm.

RESULTS

Differences among Plant Species in the Biosynthesis and Accumulation of MV and DV Pchlide in Darkness. The results of a survey comprising four monocot and nine dicot species are reported in Table I and in Figures 1 and 2. In Figures 1 and 2, the MV and DV Pchlide contents are reported in absolute amounts. In order to compensate for tissue variability, they are also reported as percentages of the total Pchlide pools.

At the beginning of the dark period (0 h darkness), DV Pchlide was predominant in all plant species and ranged from 72% of the total Pchlide pool in bean to 97% in oat (Table I). Total Pchlide and DV Pchlide levels increased throughout the dark phase of the photoperiod in all species examined. However, three different patterns of change in the DV Pchlide pool were observed.

One type of response to darkness was observed in dicots such as purslane, mustard, and cucumber (Table I, Fig. 1). These plants were characterized by a DV Pchlide accumulation which was accompanied by varying levels of MV Pchlide formation, throughout the 10 h of darkness. At all times during that period, the DV Pchlide content remained higher than that of MV Pchlide. The DV Pchlide content of cucumber leaves and cotyledons started to decline after an abnormally long sojourn (16 to 18 h) in darkness. As a consequence, MV Pchlide became the major constituent of the Pchlide pool in cucumber after prolonged exposure to darkness (Fig. 1). It was not determined whether other plant species in this group, such as purslane and mustard, behaved in a similar manner in prolonged darkness.

A second pattern of change in the DV Pchlide pool during

Table I. *Changes in the MV and DV Pchlide Contents of Some Photoperiodically Grown Angiosperms during Darkness*

Total, MV and DV Pchlide contents were measured during darkness, starting at the end of a 14-h light period. Plants were grown under a photoperiod of 14 h light/10 h dark.

Plant Material	Age of Plants		Time in Darkness (*h*)		
			0	0.5	10
	d		*nmol/100 mg protein*		
Purslane (leaves)	27	Total	0.00[a]	3.44	6.32
		MV	(19%)	1.13	1.38
		DV	(81%)	2.31	4.94
Mustard (cotyledons + leaves)	16	Total	3.04	4.16	6.47
		MV	0.17	0.40	1.51
		DV	2.87	3.76	4.96
Bean (leaves)	7	Total	0.83	6.03	10.33
		MV	0.23	3.00	9.58
		DV	0.60	3.03	0.75
Cotton (cotyledons + leaves)	6	Total	1.49	7.94	19.54
		MV	0.25	2.80	17.80
		DV	1.24	5.14	1.74
Wheat (leaves)	6	Total	2.46	5.46	14.76
		MV	0.19	2.81	14.54
		DV	2.27	2.65	0.22
Pigweed (cotyledons + leaves)	16	Total	3.76	4.92	5.05
		MV	0.67	1.94	4.56
		DV	3.09	2.98	0.49
Soybean (cotyledons)	6	Total	0.56	1.26	12.73
		MV	0.13	0.67	12.19
		DV	0.43	0.59	0.54
Soybean (leaves)	6	Total	8.09	9.70	17.53
		MV	2.09	6.21	16.99
		DV	6.00	3.49	0.54
Oat (leaves)	6	Total	6.21	6.40	11.94
		MV	0.20	3.69	11.23
		DV	6.01	2.71	0.71
Corn (leaves)	6	Total	4.04	13.10	28.40
		MV	0.41	11.05	28.40
		DV	3.63	2.05	0.00
Lambsquarters (cotyledons + leaves)	16	Total	1.92	2.61	9.96
		MV	0.08	1.13	9.44
		DV	1.84	1.48	0.52
Tomato (cotyledons + leaves)	16	Total	4.45	3.03	11.50
		MV	0.41	0.95	10.59
		DV	4.04	2.08	0.91

[a] In this case, total Pchlide levels were too low to detect at room temperature, but the relative quantities of MV and DV Pchlide were readily determined from the 77 K fluorescence spectra.

darkness was exhibited by dicots such as cotton and bean, and by monocots such as wheat and barley. In these species, DV Pchlide accumulation underwent a transient rise during the first 30 to 60 min in darkness. This was followed by a decline in DV Pchlide content, so that by the end of 10 h in darkness, the Pchlide pool consisted mainly of MV Pchlide (Table I, Fig. 2).

A third type of response to darkness was exhibited by dicots such as soybean, tomato, lambsquarters, and pigweed and by monocots such as oat and maize. In these plants, the DV Pchlide content either remained constant (soybean cotyledons) or underwent a steady decline (the other species). By the end of 10 h in darkness, the Pchlide pool consisted mainly of MV Pchlide. Thus, all of the increase in Pchlide content was accounted for by the biosynthesis and accumulation of MV Pchlide (Table I). It was not determined whether plants of this group experienced a transient rise in DV Pchlide content during the first few min of darkness.

Differences among Plant Species in the Biosynthesis and Ac-

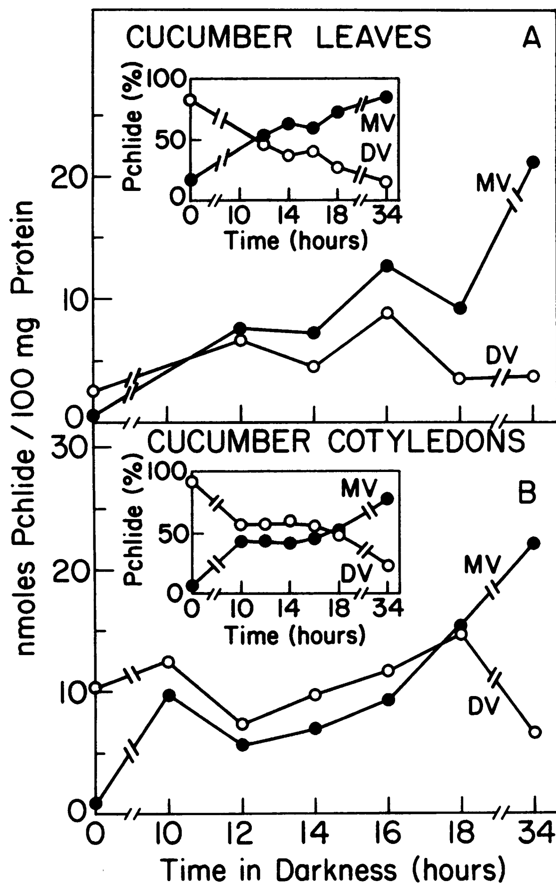

FIG. 1. Changes in the MV and DV Pchlide content in (A) 14-d-old photoperiodically grown cucumber leaves and (B) in 6-d-old cucumber cotyledons during darkness. (●), MV Pchlide; (○), DV Pchlide. The inserts depict the levels of MV and DV Pchlide as percentages of the total Pchlide content. The dark treatment was initiated at the end of the 14-h light cycle. The photoperiod consisted of 14 h light/10 h dark.

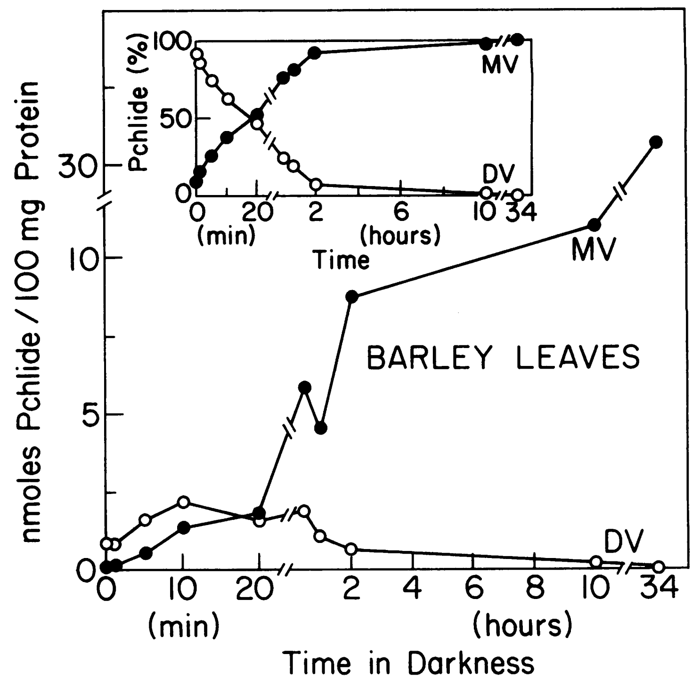

FIG. 2. Changes in the MV and DV Pchlide content in 6-d-old photoperiodically grown barley leaves during darkness. (●), MV Pchlide; (○) DV Pchlide. All other conditions are as in Figure 1.

cumulation of MV and DV Pchlide in the Light. Since various plant species differed markedly in the proportions of MV and DV Pchlide that they accumulated after 10 h in darkness, we wondered whether there would also be differences among plant species in their MV and DV Pchlide biosynthetic capabilities during the first few hours of daylight. Since we lacked the experimental facilities for simulating precisely the gradual increase and variation in light intensity and quality which take place following daybreak, the plants were illuminated with 100 ft-c of phototransforming white fluorescent light. This light intensity approximated the average insolation during the first 60 to 80 min following daybreak on a summer day. The results of these investigations are presented in Table II and in Figure 3.

In all cases, total Pchlide levels were highest at the end of the dark period (0 h light) and fell rapidly after 30 min in the light. This was expected as a result of the photoconversion of the dark-accumulated Pchlide to Chlide *a*. Furthermore, differences in the pattern of MV and DV Pchlide metabolism in low light were observed among plant species.

One type of response to low illumination was observed in cucumber cotyledons. Cucumber belonged to the group of plants that had accumulated DV Pchlide throughout the dark phases (10 h) of photoperiodic growth. As depicted in Figure 3A, the MV Pchlide content underwent a steady decline during 4 h of illumination. On the other hand, after an initial decline in DV Pchlide levels during the first 30 min of illumination, probably due to photoconversion to Chl(ide) *a*, the DV Pchlide content underwent an increase both in absolute and in relative amounts (Fig. 3A). Such an increase in Pchlide synthesis under low illumination has also been observed by others (12). By the end of 2 h of illumination, DV Pchlide constituted about 90% of the Pchlide pool. Cucumber cotyledons that were left in darkness for 17 h instead of the usual 10 h behaved in essentially the same way. During prolonged darkness, the MV Pchlide content had become higher than that of DV Pchlide (Fig. 1B, Table II). It was not determined whether purslane and mustard behaved in a similar manner to cucumber in the light.

A second type of response was exhibited by barley, bean, and soybean leaves and by soybean cotyledons. These plants had accumulated mainly MV Pchlide instead of DV Pchlide in darkness (Fig. 2, Table I) and exhibited either a transient rise in DV Pchlide content (barley and bean) or exhibited a steady level or decline in DV Pchlide content (soybean leaves and cotyledons). In all the species that were examined, the MV Pchlide level fell rapidly during the first 30 min of illumination, probably as a consequence of conversion to Chl(ide) *a*; thereafter, the MV Pchlide level fell more slowly. On the other hand, the DV Pchlide level rose rapidly during the first 30 min of illumination and more slowly thereafter. Various plant species differed in the length of time it took their DV Pchlide content to rise above that of MV Pchlide. This was achieved after about 30, 45, 51, and 214 min in bean leaves, barley leaves, soybean leaves, and soybean cotyledons, respectively.

DISCUSSION

The Pchlide pool which accumulates at night in photoperiodically grown plants most probably contributes to Chl formation at daybreak. The evidence for this is as follows: (a) in this study, the total Pchlide content, which had increased during the dark phases of photoperiodic greening, decreased significantly during the first 30 min of illumination (Table II, Fig. 3); (b) Pchlide has been reported to be photoconverted to Chlide *a* in photoperiodically grown cucumber by a brief light treatment administered at the beginning of a light cycle (8); (c) [^{14}C]Pchlide formed from

Table II. *Changes in the MV and DV Pchlide Contents of Some Photoperiodically Grown Angiosperms under Low Illumination*

Total, MV, and DV Pchlide contents were measured under 100 ft-c of white fluorescent light starting at the end of the indicated dark period. Plants were grown under a photoperiod of 14 h light/10 h dark unless otherwise indicated.

Plant Material	Age of Plants		Time in the Light (*h*)				
			0	0.5	1	2	4
	d		*nmol/100 mg protein*				
Cucumber cotyledons after 17 h of darkness	5	Total	19.02	6.66	8.02	7.58	5.82
		MV	11.41	2.52	2.15	1.66	0.67
		DV	7.61	4.14	5.87	5.92	5.15
Bean leaves after 10 h of darkness	7	Total	10.63	6.13	9.45	7.75	8.52
		MV	9.63	3.16	3.26	2.43	2.41
		DV	1.00	2.97	6.19	5.32	6.11
Soybean cotyledons after 10 h of darkness	6	Total	2.88	1.83	1.20	1.02	1.06
		MV	2.84	1.28	0.85	0.60	0.51
		DV	0.04	0.55	0.35	0.42	0.55
Soybean leaves after 10 h or darkness	6	Total	16.56	11.04	10.59	8.17	9.37
		MV	16.17	6.21	5.07	3.30	2.97
		DV	0.39	4.83	5.52	4.87	6.40

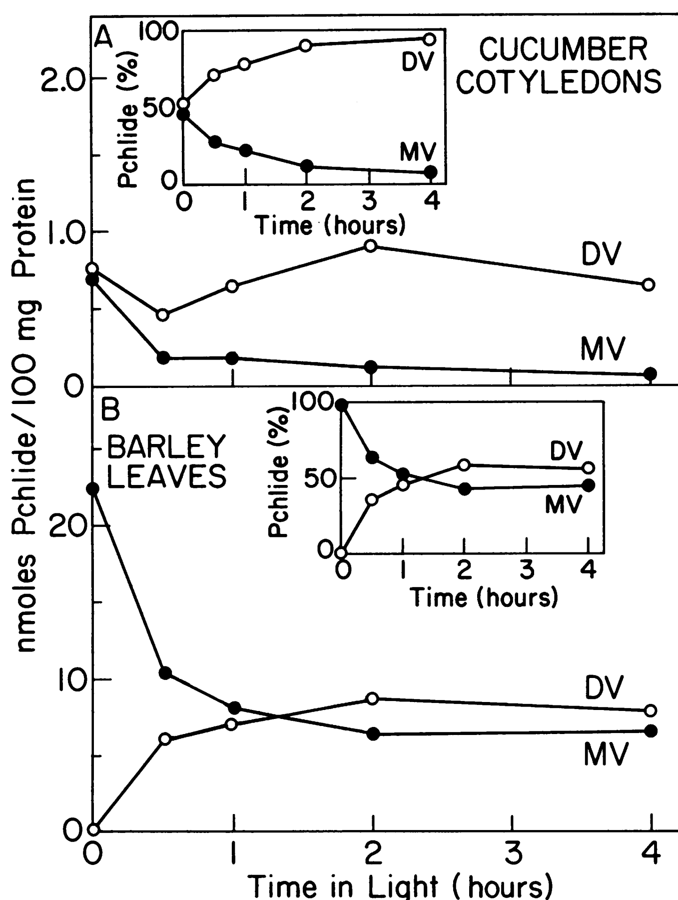

FIG. 3. Changes in the MV and DV Pchlide content in (A) 7-d-old photoperiodically grown cucumber cotyledons and (B) 4-d-old barley leaves under low illumination. (●), MV Pchlide; (○) DV Pchlide. The low intensity (100 ft-c) was meant to simulate the average insolation during the first 60 to 80 min following daybreak. The light treatment was initiated at the end of the 10-h dark cycle. All other conditions are as in Figure 1.

[^{14}C]ALA in green cucumber seedlings (C. A. Rebeiz, unpublished data) or from $^{14}CO_2$ in green barley seedlings (24), was converted to [^{14}C]Chl *a* in the light. Since the MV and DV Pchlide content varied considerably among plant species at the end of the dark period (10 h), it is logical to surmise that the contribution of MV Pchlide and DV Pchlide to Chl formation at daybreak also varied among species. This hypothesis is in turn supported by the demonstrated photoconvertibility of MV and DV Pchlide to MV and DV Chlide *a*, respectively, in etiolated and greening barley, corn, bean, and cucumber seedlings (4, 5, 9).

Over and beyond the species-dependent differential contribution of MV and DV Pchlide to Chl formation at daybreak looms the question of the biochemical origin of this phenomenon. At this stage, two extreme possibilities can be envisaged. With strict adherence to the traditional single-branched pathway of Chl biosynthesis (13), one might hold that the decrease in DV Pchlide and increase in MV Pchlide content was due to the conversion of DV Pchlide to MV Pchlide. Likewise, the increase in DV Pchlide content might be considered to result from an inhibition of DV Pchlide conversion to MV Pchlide in darkness and/or from a rapid conversion of MV Pchlide to Chlide *a* in the light. None of these hypotheses appears to be tenable. Indeed, it has been impossible to demonstrate the conversion of DV Pchlide to MV Pchlide in potent cell-free systems (B. C. Tripathy, C. A. Rebeiz, unpublished data).

A better interpretation of the data can be made within the conceptual framework of a multibranched Chl biosynthetic pathway (15, 16, 23). Such an interpretation would hold that light and darkness control and regulate the rates of Chl formation via separate MV and DV Chl biosynthetic routes. This in turn: (a) is compatible with the proposal that the function of separate Chl biosynthetic routes is to control and regulate the orientation of specific Chl molecules at specific sites in the thylakoid membranes (6, 17, 23); and (b) is also compatible with the demonstration of separate and independent MV and DV Pchlide biosynthetic routes which originate in MV and DV protoporphyrin IX. These two biosynthetic routes were recently demonstrated *in toto* in cell-free systems prepared from greening monocots and dicots (B. Tripathy and C. A. Rebeiz, unpublished data). What is not accounted for by the above hypothesis is the fate of the transient DV Pchlide which is formed in barley, wheat, cotton, and bean during the initial stages of darkness. We are presently

investigating the possibility that this DV Pchlide may be converted to Chlide *a* in darkness (1).

Finally, we have recently reported that ALA-induced tetrapyrrole accumulation in green plants can cause extensive photodynamic damage to some plant species, while other plant species remain unaffected (21). Cucumber cotyledons, soybean leaves, and common bean leaves were susceptible to ALA-induced photodynamic damage while soybean cotyledons and barley leaves were not. Under the subdued light levels used in this study, the nonsusceptible plants species maintained higher relative levels of MV Pchlide than the susceptible ones. The relationship of the susceptibility of various plant species to photodynamic damage caused by ALA-induced MV and DV tetrapyrrole accumulation under high light intensities is presently under investigation.

Acknowledgement—The authors wish to thank Elsje Smit for assistance in the preparation of this manuscript.

LITERATURE CITED

1. Adamson H, N Packer 1984 Dark synthesis of chlorophyll *in vivo* and dark reduction of chlorophyll *in vitro* by pea chloroplasts. *In* C Sironval, M Brouers, eds, Protochlorophyllide Reduction and Greening. Nijhoff/Junk The Hague, pp 353–363
2. Belanger FC, CA Rebeiz 1979 Chloroplast biogenesis 27. Detection of novel chlorophyll and chlorophyll precursors in higher plants. Biochem Biophys Res Commun 88: 365–372
3. Belanger FC, CA Rebeiz 1980 Chloroplast biogenesis. Detection of divinyl and monovinyl protochlorophyllide in higher plants. J Biol Chem 255: 1266–1272
4. Belanger FC, CA Rebeiz 1980 Chloroplast biogenesis 30. Chlorophyll(ide) (E459F675) and chlorophyll(ide) (E449F675) the first detectable products of divinyl and monovinyl protochlorophyll photoreduction. Plant Sci Lett 18: 343–350
5. Belanger FC, JX Duggan, CA Rebeiz 1982 Chloroplast biogenesis. Identification of chlorophyllide *a* (E458F674) as a divinyl protochlorophyllide *a*. J Biol Chem 257: 4849–4858
6. Belanger FC, CA Rebeiz 1984 Chloroplast biogenesis 47. Spectroscopic study of net spectral shifts induced by axial ligand coordination in metalated tetrapyrroles. Spectrochim Acta 40A: 807–827
7. Cohen CE, MB Bazzaz, SH Fullett, CA Rebeiz 1977 Chloroplast Biogenesis XX. Accumulation of porphyrin and phorbin pigments in cucumber cotyledons during photoperiodic greening. Plant Physiol 60: 743–746
8. Cohen CE, CA Rebeiz 1978 Chloroplast biogenesis XXII. Contribution of short wavelength and long wavelength protochlorophyll species to the greening of higher plants. Plant Physiol 61: 824–829
9. Duggan JX, CA Rebeiz 1982 Chloroplast biogenesis 37. Induction of chlorophyllide *a* (E459F675) accumulation in higher plants. Plant Sci Lett 24: 27–37
10. Duggan JX, CA Rebeiz 1982 Chloroplast biogenesis 42. Conversion of divinyl chlorophyllide *a* to monovinyl chlorophyllide *a in vivo* and *in vitro*. Plant Sci Lett 27: 137–145
11. Hanamoto CM, PA Castelfranco 1983 Separation of monovinyl and divinyl protochlorophyllides from etiolated and phototransformed cucumber cotyledons. Plant Physiol 73: 79–81
12. Henningsen KW, JE Boynton 1970 Macromolecular physiology of plastids VIII. Pigment and membrane formation in plastids of barley greening under low light intensity. J Cell Biol 44: 290–304
13. Jones OTG 1979 Chlorophyll biosynthesis. *In* D Dolphin, ed, The Porphyrins, Vol 6. Academic Press, New York, pp 179–232
14. Koski VM, CS French, JHC Smith 1951 The action spectrum for the transformation of protochlorophyll to chlorophyll *a* in normal and albino corn seedlings. Arch Biochem Biophys 31: 1–17
15. Rebeiz CA 1982 Chlorophyll: anatomy of a discovery Chemtech 12: 52–63
16. Rebeiz CA, J Lascelles 1982 Biosynthesis of pigments in plants and bacteria. *In* Govindjee, ed, Photosynthesis: Energy Conversion in Plants and Bacteria, Vol 1. Academic Press, New York, pp 699–780
17. Rebeiz CA, FC Belanger 1984 Chloroplast biogenesis 46. Calculation of net spectral shifts induced by axial ligand coordination in metalated tetrapyrroles. Spectrochim Acta 40A: 793–806
18. Rebeiz CA, PA Castelfranco 1965 Fractionation and properties of an extramitochondrial enzyme system from peanuts catalyzing the β-oxidation of palmitic acid. Plant Physiol 40: 281–285
19. Rebeiz CA, H Daniell, JR Mattheis 1982 Chloroplast bioengineering: the greening of chloroplasts *in vitro*. *In* C Scott, ed, 4th Symposium on Biotechnology in Energy Production and Conservation. John Wiley, New York, pp 413–439
20. Rebeiz CA, FC Belanger, CE Cohen, SA McCarthy 1979 The greening of higher plants. Ill Res 21: 3–4
21. Rebeiz CA, A Montazer-zouhoor, HJ Hopen, SM Wu 1984 Photodynamic herbicides 1. Concept and phenomenology. Enzyme Microb Technol 6: 390–401
22. Rebeiz CA, JR Mattheis, BB Smith, CC Rebeiz, DF Dayton 1975 Chloroplast biogenesis. Biosynthesis and accumulation of protochlorophyll by isolated etioplasts and developing chloroplasts. Arch Biochem Biophys 171: 549–567
23. Rebeiz CA, SM Wu, M Kuhajda, H Daniell, EJ Perkins 1983 Chlorophyll *a* biosynthetic routes and chlorophyll *a* chemical heterogeneity in plants. Mol Cell Biochem 57: 97–125
24. Shlyk AA 1965 Chlorophyl metabolism in green plants. Translated by United States Atomic Energy Commission, pp 293
25. Tripathy BC,CA Rebeiz 1985 Chloroplast biogenesis: quantitative determination of monovinyl and divinyl Mg-protoporphyrins and protochlorophyll(ides) by spectrofluorometry. Anal Biochem. In press
26. Wolff JB, L Price 1957 Terminal steps of chlorophyll *a* biosynthesis in higher plants. Arch Biochem Biophys 72: 293–301
27. Wu SM, CA Rebeiz 1984 Chloroplast biogenesis 45. Molecular structures of protochlorophyllide (E443F625) and of chlorophyllide *a* (E458F674). Tetrahedron 40: 659–664