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Protochlorophyllide Resynthesis in Dark-Grown Bean Leaves[1]

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Abstract. **The protochlorophyllide content of dark-grown bean leaves was determined at various ages. It was detectable the second day after germination and reached a maximum on about the tenth day.**

The resynthesis of protochlorophyllide following a single irradiation and the rate of the 685 to 673 mμ shift of the newly formed chlorophyll was examined. Leaves up to 5-days old show no lag phase in protochlorophyllide resynthesis. By the sixth day, a lag phase is evident which increases with age. The rate of the 685 to 673 mμ shift is similarly age dependent.

In the leaf, a dark synthesis of protochlorophyllide follows the phototransformation of the pigment to chlorophyll. The time course of the process was examined by several workers. Liro (1), Scharfnagel (2), and Virgin (3) found no lag phase in the dark synthesis. Shibata (4) and Butler (5) noted a lag phase in the dark synthesis which was dependent on the age of the leaf. Madsen (6) and Augustinussen and Madsen (7) reported a lag phase in the resynthesis.

A systematic examination of the time course of protochlorophyllide resynthesis following illumination and the spectral shifts of newly formed chlorophyll were made on dark-grown bean leaves. The age of the tissue has a profound influence on many aspects of protochlorophyllide and the newly formed chlorophyll, and these results are presented here.

Materials and Methods

Bean plants, *Phaseolus vulgaris* CV. Red Kidney, were grown in vermiculite in complete darkness at 25°. All operations were performed under green safelights. The phototransformation of protochlorophyllide to chlorophyll was achieved using a 150 W internal reflector projection lamp with a 6 cm water filter. The plant material was held 30 cm from the source for 1 minute.

Protochlorophyllide resynthesis was determined either *in vivo* or by extraction of the pigment. Measurements *in vivo* were made by fastening 2 superimposed etiolated leaves between transluscent mending tape to a piece of light cardboard with an aperature slightly larger than the beam of the spectrophotometer. A similar aperature was used in the reference beam. Opal glass (4) was placed in both the sample and reference beams of the Cary 14 spectrophotometer. Protochlorophyllide resynthesis *in vivo* was monitored by measuring the 650 mμ absorbance increase with time. Protochlorophyllide was extracted from 1 g fresh weight samples of leaves by grinding in a mortar with 4 ml of acetone and then 80 % acetone, filtered and made to final volume of 20 ml. The pigment was transferred to ether and absorbances measured. The pigment concentrations were calculated using the equations of Koski (8).

Results

The protochlorophyllide content of dark-grown bean leaves of various ages was examined. The pigment content is low in very young leaves, increases rapidly to a maximal value and then remains constant (fig 1). The dark resynthesis of proto-

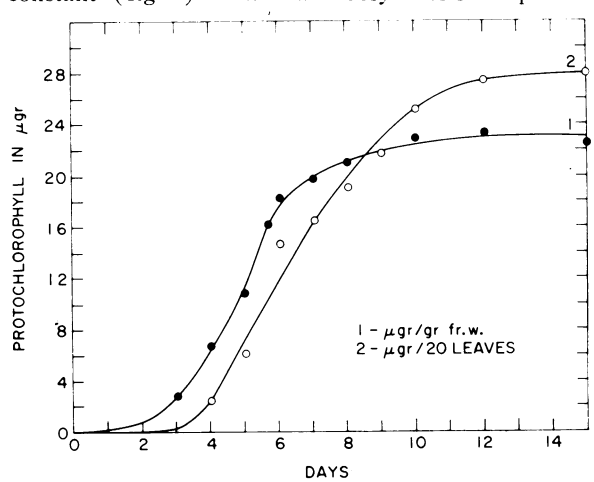

FIG. 1. Protochlorophyllide content of dark-grown bean leaves at various ages on a per gram fresh weight or per 20 leaves basis.

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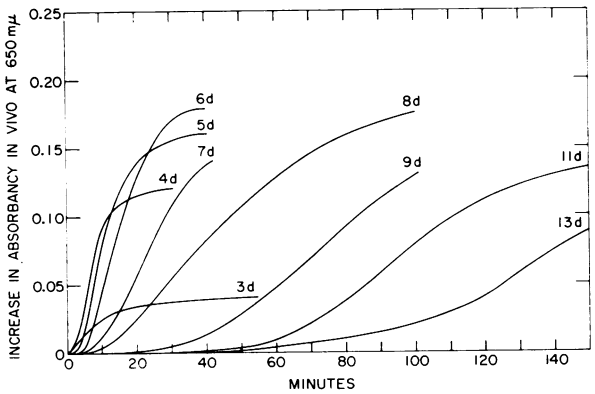

FIG. 2. Time course of protochlorophyllide resynthesis in darkness following a single 1 minute illumination in dark-grown bean leaves. The data was obtained by measuring the increase in absorbance at 650 mμ of 2 thicknesses of leaves by the opal glass method of Shibata (4).

chlorophyllide following illumination was examined in dark-grown bean leaves of various ages. The length of the lag period, the rate of resynthesis, and the final concentration of resynthesized protochlorophyllide are markedly age dependent. These results are based on data obtained from the in vivo increase in absorption at 650 mμ (fig 2) and the content of protochlorophyllide determined by extraction of the pigment (fig 3).

The phototransformation of protochlorophyllide and the subsequent dark transformations of the newly formed chlorophyll were examined in dark-grown bean leaves at various ages. In the 5-day-old leaves, the dark transformations of the newly formed chlorophyll were rapid and the resynthesis of protochlorophyllide is obvious 5 minutes after illumination from the absorbance increase at 650 mμ (fig 4). The rate of the dark transformations of the newly formed chlorophyll and the resynthesis

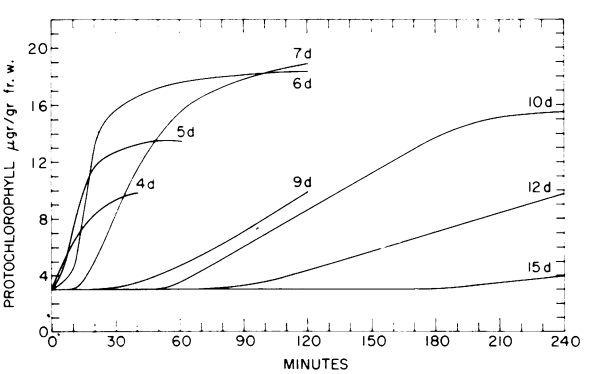

FIG. 3. Time course of protochlorophyllide resynthesis in darkness following a single 1 minute illumination in dark-grown bean leaves. The pigment was extracted from the leaves. The non-transformable protochlorophyllide after illumination is 3.0 ± 0.3 μg/g fresh weight.

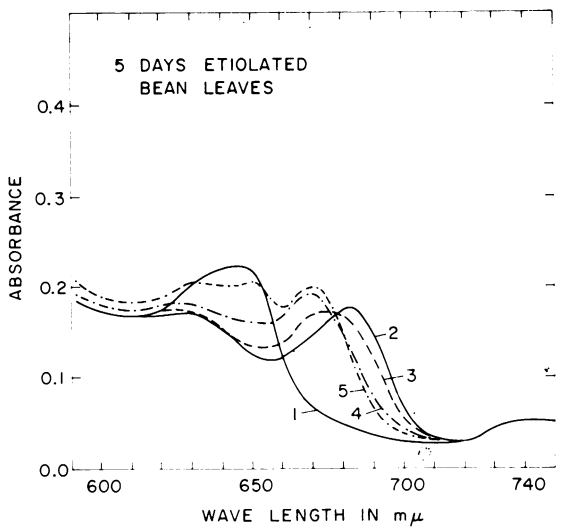

FIG. 4. Absorption spectra changes of 2 thicknesses of 5-day old dark-grown bean leaves before and after a single 1 minute illumination. 1- before illumination, 2, 3, 4, and 5- after 0, 2, 5, and 10 minutes following illumination respectively.

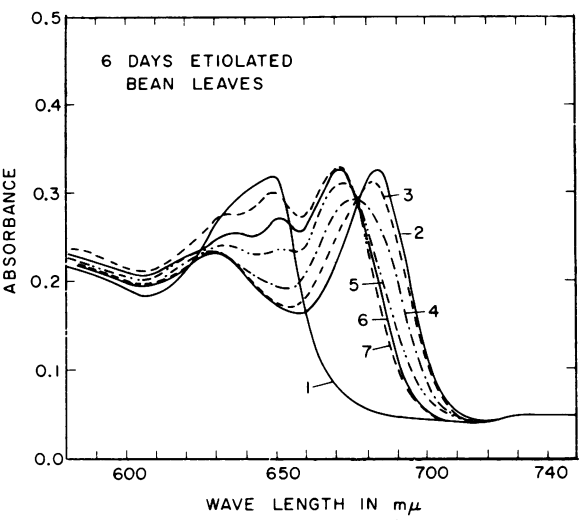

FIG. 5. Absorption spectra changes of 2 thicknesses of 6-day old dark-grown bean leaves before and after a single 1 minute illumination. 1- before illumination, 2, 3, 4, 5, 6, and 7- after 0, 5, 10, 15, 20, and 28 minutes following illumination respectively.

of protochlorophyllide are appreciably slower in the 6 and 9-day-old dark-grown bean leaves (figs 5 and 6).

Discussion

The protochlorophyllide content of dark-grown bean leaves at various ages is shown in figure 1. There is an initial period up to the fourth day of low protochlorophyllide content followed by period

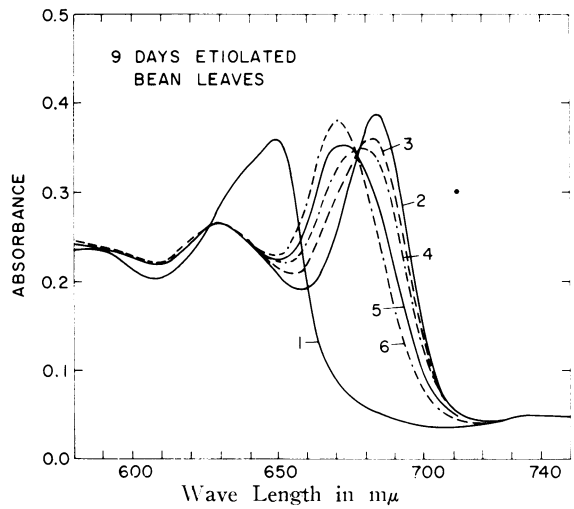

FIG. 6. Absorption spectra changes of 2 thicknesses of 9-day old dark-grown bean leaves before and after a single 1 minute illumination. 1- before illumination, 2, 3, 4, 5, and 6, after 0, 10, 15, 20, and 35 minutes following illumination respectively.

of linear increase. The protochlorophyllide content reaches a maximum and then remains nearly constant. These results are similar but not identical when they are based on fresh weight or on a per leaf unit. One gram fresh weight of dark-grown bean leaves consists of about 200 leaves at 3 days, 80 leaves at 4 days, 38 leaves at 5 days, or 24 leaves at 6 days of age. The number of leaves per gram remains nearly constant after 6 days. Protochlorophyllide is present in 2-day-old dark-grown leaves, and chlorophyll will accumulate on illumination indicating the presence of the entire protochlorophyllide synthesizing system.

The resynthesis of protochlorophyllide measured either *in vivo* or after extraction shows a similar time course. There is no lag in resynthesis through the fifth day. Beginning with the sixth day, there is a lag period which increases in length with age. Both Shibata (4) and Butler (5) examined protochlorophyllide resynthesis in dark-grown bean leaves of various ages. They noted that the lag phase of resynthesis was age dependent. However, the youngest leaves they examined were 7 and 6 days of age, respectively. Although Shibata (4) classified 9 to 10 day old leaves as normal, it is apparent that the rate of protochlorophyllide synthesis is considerably diminished at this age.

The absorption spectra changes following a single illumination of dark-grown bean leaves were fully described by Shibata (4) and confirmed by Butler (5). The newly formed chlorophyll which absorbs maximally at about 686 mμ (C684) shifts in darkness to a form absorbing at about 673 mμ (C673). Butler (5) noted that the C684 to C673 shift was faster in 6- than 9-day-old leaves. Examination of the absorption shifts of the 5-day-old leaves shows that they are more rapid than in 6- or 9-day-old leaves. The resynthesis of protochlorophyllide is also faster as seen from the 650 mμ absorbancy increase.

Sisler and Klein (9) found that the time course of chlorophyll synthesis in dark-grown bean leaves on illumination was markedly dependent on age. Although they did not measure protochlorophyllide, the age effect is likely related, at least in part, to the capacity for protochlorophyllide synthesis. In examination of protochlorophyllide synthesis and the dark absorption shifts of newly-formed chlorophyll, the age of the leaf must be considered as an important variable. Studies on chlorophyll phytolization and chloroplast structural development may also be influenced by the physiological age of the tissue.

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