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Photosynthetic Studies on a Pea-mutant Deficient in Chlorophyll

H. R. Highkin,[2] N. K. Boardman, and D. J. Goodchild
Division of Plant Industry, CSIRO, Canberra, A.C.T., Australia

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Abstract. A chlorophyll-deficient mutant of pea (*Pisum sativum*) was found as a spontaneous mutation of the variety Greenfeast. Total chlorophyll of the mutant leaves was about one-half that of normal pea leaves per mg dry weight, and the ratio of chl *a*:chl *b* ranged from 10 to 18, compared with 3 for normal pea. In each generation the mutant plants gave rise to normal and mutant plants and lethal plants with yellow leaves.

For a normal pea plant, CO_2 uptake was saturated at about 60,000 lux, whereas with mutant leaves, the rate of CO_2 uptake was still increasing at 113,000 lux. At 113,000 lux the mutant and normal leaves showed similar rates of CO_2 fixation per unit area of leaf surface, but on a chlorophyll basis the mutant leaves were twice as active. Hill reaction measurements on isolated chloroplasts also showed that the mutant chloroplasts were saturated at higher intensities than the normal, and that the activity of the mutant was at least double that of the normal on a chlorophyll basis.

It is suggested that the photosynthetic units of the mutant chloroplasts contain about half the number of chlorophyll molecules as compared to the normal photosynthetic units.

Electron microscopy of leaf sections of normal and mutant leaves showed that the mutant chloroplasts contain fewer lamellae per chloroplast and fewer lamellae per granum. The lethal chloroplasts, which were virtually devoid of chlorophyll, were characterized by an absence of grana.

Previous studies with a mutant of barley (*Hordeum vulgare* L.) devoid of chlorophyll *b* indicated that chlorophyll *b* is not essential for photosynthetic activity in a higher plant (6, 11, 13). Photosynthetic rates obtained with the mutant plants did not differ significantly from those obtained in normal plants (13). Hill reaction measurements on isolated chloroplasts showed that the mutant chloroplasts were more active per mg of total chlorophyll if assayed at saturating light intensities, but less active at low light intensities (6). The mutant chloroplasts were less fluorescent (7), and their molar ratio of total chlorophyll to cytochrome b_6 plus cytochrome 559 was only about one-half of the corresponding ratio of normal chloroplasts (4).

It was concluded from these studies that the mutant chloroplasts have less chlorophyll in their light-harvesting assemblies than the normal chloroplasts, but comparable amounts of the components of the photosynthetic electron transport chain (4). It was further suggested that the mutant chloroplasts have a decreased amount of chlorophyll in photosystem 2, relative to photosystem 1 (7). This would result in photosystem 2 absorbing a lower fraction of the incident quanta and would account for the lower photochemical efficiency of the mutant chloroplasts at low light intensities.

Electron microscopy of mutant leaf sections showed fewer grana in the mutant chloroplasts and fewer lamellae per granum, in comparison with the normal chloroplast (8).

In the present studies, we have investigated the photosynthetic properties of a pea mutant deficient in both chlorophyll *a* and chlorophyll *b*, and examined the fine structure of its chloroplasts. The level of total chlorophyll is shown to be lower in the pea than in the barley mutant, and a study of its photosynthetic properties enables a useful comparison with the barley mutant, and the chlorophyll-deficient tobacco mutants of Schmid and Gaffron (18, 19) and Homann and Schmid (14). A study of the relationship between photosynthetic activity and structure in such chlorophyll-deficient mutants may assist in the elucidation of the molecular organization of the chloroplast. A summary of this work was presented previously (12).

Materials and Methods

Plant Material. The mutant pea was derived from a commercial variety (*Pisum sativum*, var.

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[2] Present address: Department of Biology, San Fernando Valley State College, Northridge, California.

Greenfeast) as a spontaneous mutation. The mutant was observed in a population of plants growing in artificial light at a temperature of 21°/19° and a photoperiod of 16 hr. It was distinguishable from the other plants by the pale-green color of its leaves. The mutant was grown to maturity, and the seed saved and grown for a second generation. Seeds from each of these plants were harvested and grown for a third generation, and this procedure was repeated for a number of generations to date. After the first generation, the plants were grown in a controlled temperature glasshouse of the CSIRO phytotron in a 16 hr photoperiod and a temperature of 21°/19°. Normal pea plants were grown under identical conditions.

Preparation of Chloroplasts. Leaves (3–5 g fresh wt) either from mutant or normal plants were ground in a mortar, chilled in ice, with 15 ml of 0.05 M phosphate buffer (pH 7.2), containing 0.3 M sucrose and 0.01 M KCl. The resulting brei was filtered through 2 layers of Miracloth, and the chloroplasts sedimented by centrifugation at 1000*g* for 10 min. The chloroplasts were resuspended in the sucrose-phosphate buffer (30 ml) and again centrifuged at 1000*g* for 10 min. Chloroplasts obtained from 3 g of mutant leaves were resuspended in 3 ml of sucrose-phosphate buffer and those from 3 g of normal leaves were resuspended in 6 ml.

Chlorophyll Determinations on Chloroplasts or Leaves. Total chlorophyll was determined in 80 % acetone with a Cary recording spectrophotometer, using the equations of Arnon (2). For determinations with whole leaves 1.0 g fresh wt samples were ground in 80 % acetone in a Servall Omnimixer. The residue obtained after centrifugation was extracted with 80 % acetone in the Omnimixer, and the procedure repeated. The 3 supernatants were combined and made up to 250 ml. Dry weights were determined after heating samples at 80° until a constant weight was achieved, usually 24 hr. Leaf areas were obtained by outlining leaves on mm paper.

Chl *a*:chl *b* ratios of mutant leaves or mutant chloroplasts were determined in ethyl alcohol by a sensitive spectrofluorimetric method, full details of which will be published elsewhere. Briefly, the ethanol extracts were diluted to an absorbancy of 0.2 at 436 nm, and cooled to the temperature of liquid nitrogen. Fluorescence emission spectra were recorded on a fluorescence spectrometer incorporating automatic correction for photomultiplier and monochromator responses, and variation in energy output of the light source (7). The extract was excited at 474 nm, the wavelength maximum for chlorophyll *b*, and the chl *a*:chl *b* ratio determined from the relative amplitudes of the fluorescence emission at 680 nm (Chl *a*) and 656 nm (Chl *b*). The method was calibrated with mixtures of purified chlorophyll *a* and chlorophyll *b*.

Absorption spectra of intact leaves at 77°K were recorded on a Cary Model 14R spectrophotometer, equipped with a scattered transmission accessory (6).

Carbon Dioxide Fixation. Carbon dioxide uptake by leaves of mutant and normal plants was determined with an infra-red gas analyzer (Grubb Parsons). A leaf attached to the plant was enclosed in a perspex airtight container, and an air sample containing 300 ppm of CO_2 was pumped across the leaf at a rate of 2000 cc/min. The leaf was illuminated with 4 Mazda FBRU 1000 watt mercury vapor high pressure lamps, and the light intensity was varied with neutral grey "Sarlon" shade cloth. Light intensities were measured with a undirectional Kipp thermopile and also with a selenium light meter (Eel Instruments). The temperature of the leaves was determined with a thermocouple placed under an identical set of leaves in the same cabinet as the experimental leaves. The temperature of the cabinet was 17.5°.

Photochemical Activities of Chloroplasts. Hill reaction measurements were made as described previously (1), except that ferricyanide reduction was measured directly in a Cary spectrophotometer by the absorbance decrease at 418 nm.

White-light intensities in the range of 1400 to 200,000 lux were obtained from a 250 watt photoflood lamp operated at 200 volts. The cuvette containing the reaction mixture was positioned at varying distances from the lamp. Light intensities were measured with a Weston meter, Model 603, calibrated in ft-c.

Electron Microscopy. For electron microscopy several leaf pieces, approximately 1 mm^2, were cut from the second leaf of normal, mutant or lethal pea plants and fixed in 3 % glutaraldehyde (purified by vacuum distillation) in 0.025 M phosphate buffer pH 7.2 for 1.5 hr at 20°. After washing in phosphate buffer for 1 hr the leaf pieces were post-fixed in 2 % osmium tetroxide in 0.025 M phosphate buffer pH 7.2 for 2 hr at 20°, washed in buffer, dehydrated in ethanol, and embedded in an araldite epon mixture (17). The resin was vacuum infiltrated at 85° and polymerized at the same temperature for 24 hr before sectioning with an LKB ultra-microtome. Sections were stained with saturated uranyl acetate in 50 % ethanol for 1 hr followed by Fiske's lead stain (9) before examination in a Philips EM200 electron microscope.

Electron micrographs of the chloroplasts were examined for distinctness and resolution of lamellae and 10 each of the mutant and normal were selected. The numbers of single lamellae, groups of lamellae (grana) per chloroplast, and number of lamellae per group were recorded for each chloroplast and the results analyzed statistically.

Results

Chlorophyll Content of Leaves. The pale-green leaves of the original mutant plant gave an absorp-

tion spectrum at 77° which closely resembled the spectrum published previously for chloroplasts from the barley mutant devoid of chlorophyll *b* (6). No band was detectable at 648 nm, the position of the absorption maximum of chlorophyll *b* *in vivo*. In contrast, the dark-green leaves of a normal pea plant showed a distinct band at this wavelength. The mutant pea leaves did not show a distinct band at 470 nm, which also is in agreement with the barley mutant and indicates that the mutant pea leaves have considerably less chlorophyll *b* than normal plants.

Spectrofluorimetric examination of ethanol extracts of leaves from the original mutant plant gave a chl *a*:chl *b* ratio of 20 for young leaves and 18 for physiologically older leaves. The normal pea plants had the usual chl *a*:chl *b* ratio of 3. Growth of the mutant plant was comparable to that of a normal pea plant and the yield of seed was about the same for normal and mutant plants.

In each generation it was found that the mutant plants gave rise to both normal and mutant plants, as well as lethal plants with yellow leaves. The segregation of the offspring was in an approximate ratio of 1:2:1 for normal:mutant:lethal. Genetic crosses have been made to determine more precisely the mode of inheritance of the character. The results of these studies will be published elsewhere.

The chl *a*:chl *b* ratios observed for leaves from the second, third and fourth generation were lower than for the original plant, the ratio varying from 9 to 15. Older leaves usually gave lower ratios than younger leaves from the same plant.

Chlorophyll contents of leaves from the second generation of plants are shown in table I. Leaves from young mutant plants contained about one-half of the total chlorophyll of the normal plants of the same age. The plants were sampled again after further growth for 3 weeks. The chlorophyll contents were higher by about 25 %, but the relative amounts in the normal and mutant remained about the same. The chl *a*:chl *b* ratio of mutant leaves declined from 15 at 3 to 4 weeks to 10.3 at 8 to 9 weeks.

The chlorophyll contents reported in table II were made on the individual leaves used for the CO_2-uptake studies. The mutant leaves contained slightly less than half the chlorophyll of the normal leaves on a fresh weight basis. Per unit area, the mutant leaves have 40 % of the chlorophyll of the normal leaves.

Chlorophyll was virtually absent from the leaves of the lethal plants, being barely detectable in ethanol extracts by the sensitive spectrofluorimetric method.

Carbon Dioxide Fixation. Measurements of CO_2 uptake by leaves of intact plants are shown in table III. For the normal plant, CO_2 uptake was saturated at about 60,000 lux, whereas with mutant leaves, the rate of CO_2 uptake was still increasing at 120,000 lux. Measurements of CO_2 uptake at intensities >120,000 lux were unreliable because of heating. The phytotron cabinet was maintained at 17.5°, but the temperature of the underside of a leaf increased from 23° at 3000 lux to 33° at 120,000 lux. At 120,000 lux, the mutant leaves showed a slightly

Table I. *Chlorophyll Contents of Leaves From Normal and Mutant Plants*

The mntant plants were of the second generation grown in a 16 hr photoperiod at a temperature of 21°:19° in a glasshouse and supplemented with artificial light. Age refers to time after planting the seed. The weights of the mutant and normal leaves expressed as a percentage of the fresh weight were 15.8 and 16.6 respectively. The chl *a*/chl *b* ratio of the mutant was determined by spectrofluorimetry of ethanol extracts, and total chlorophyll by spectrophotometry of acetone extracts.

	Mutant	Normal	Mutant	Normal	Mutant	Normal
Age of plant	3-4 weeks		6-7 weeks		8-9 weeks	
Chl *a* + chl *b* (mg/g dry wt)	6.05	12.0	7.33	15.2	...	...
chl *a*/chl *b*	15	2.9	...	...	10.3	2.9

Table II. *Leaf Areas and Chlorophyll Contents of Single Leaves*

The individual leaves were used for CO_2 uptake measurement before detachment from the plants. The mutant plants were from the third generation grown in a 16 hr photoperiod at a temperature of 21°:19° in a glasshouse supplemented with artificial light.

	Mutant		Normal	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Fresh weight of leaf (g)	0.230	0.388	0.311	0.458
Leaf area (cm^2)	13.0	22.5	13.3	25.1
Chl *a* + Chl *b* (μg/g fresh wt)	1040	1005	2090	2260
Chl *a* + Chl *b* ($\mu g/cm^2$)	17.5	17.3	49.0	41.2

Table III. *Carbon Dioxide Uptake by Normal and Mutant Plants*

The mutant plants were from the third generation. Plants were illuminated with 4 "Mazda" FBRU 1000 watt high pressure mercury vapor lamps and the light intensity was varied with neutral grey "Sarlon" shade cloth. Light intensities were measured with a selenium light meter, and with a Kipp thermopile. The equilibrium leaf temperature was determined with a thermopile placed beneath an identical set of leaves. CO_2 uptake was measured with an infra-red gas analyzer. Total chlorophyll was determined on acetone extracts of leaves.

Light Intensity	Equilibrium leaf temperature	CO_2 uptake					
		Mutant		Normal		Mutant Average	Normal Average
		Expt. 1	Expt. 2	Expt. 1	Expt. 2		
lux $\times 10^{-3}$		*mg* dm^{-2} hr^{-1}				μ*mole* (*mg chl*$\times$*hr*)$^{-1}$	
11.3	33	18.4	20.2	18.9	23.3	252	115
7.49	30	16.4	19.4	18.9	23.3	234	115
4.25	27.5	14.6	17.3	17.9	21.8	209	102
3.35	25.8	12.4	13.7	16.3	19.0	171	90
0.93	24	6.4	7.4	9.7	10.0	90	50
0.25	23	3.6	4.1	5.3	5.3	51	27

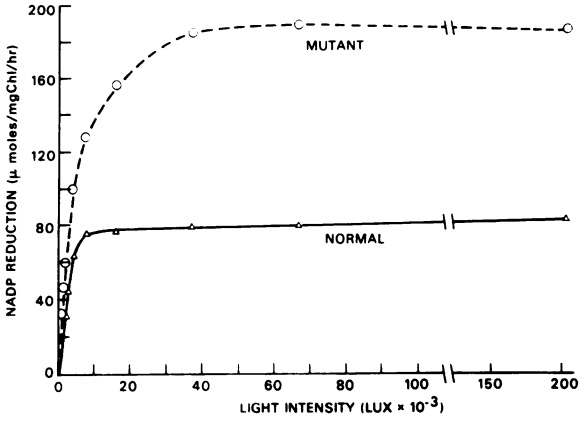

FIG. 1. Light saturation curves for the reduction of $NADP^+$ by isolated chloroplasts. The reaction mixture contained (in 3 ml), chloroplasts containing 25 μg chlorophyll (normal) or 12 μg (mutant), and (in μmoles): tris-HCl buffer (pH 8.0), 40; NaCl, 70; $MgCl_2$, 10; $NADP^+$, 0.6 and a saturating amount of spinach ferredoxin. The rate of $NADP^+$ reduction was calculated from the increase in absorbance at 340 nm, after an illumination time of 2 min.

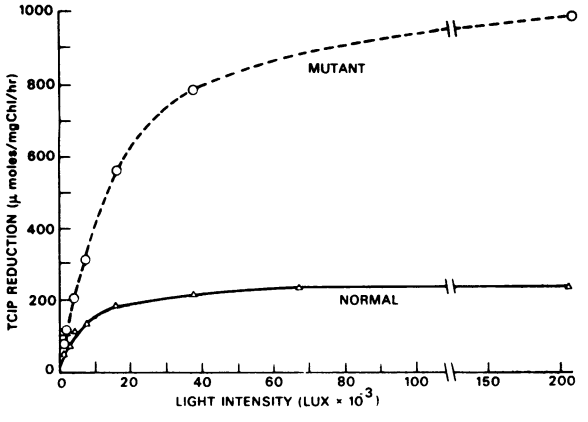

FIG. 2. Light saturation curves for the reduction of trichlorophenol indophenol by isolated chloroplasts. The reaction mixture contained (in 3 ml), chloroplasts containing 8 μg chlorophyll (normal) or 2 to 4 μg (mutant), and (in μmoles): tris-HCl buffer (pH 7.8), 40; NaCl, 70; and TCIP, 0.06. The reaction rates were measured by the decrease in absorbance at 620 nm after illumination for 45 sec or 2 min (for low light intensities).

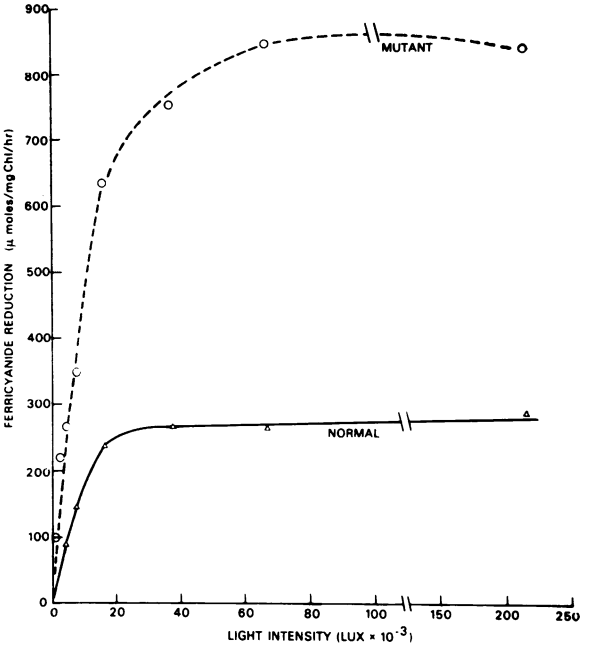

FIG. 3. Light saturation curves for the reduction of ferricyanide by isolated chloroplasts. The reaction mixture contained (in 3 ml) chloroplasts containing 25 μg chlorophyll (normal) or 12 μg (mutant), and (in μmoles): tris-HCl buffer (pH 8.0), 40; NaCl, 70; $MgCl_2$, 10; ferricyanide, 1.0. The reaction rates were measured by the decrease in absorbance at 418 nm after illumination for 2 min.

lower rate of CO_2 fixation than the normal leaves, per unit area of leaf surface, but on a chlorophyll basis the mutant leaves with a CO_2 uptake of 252 μmoles/mg chl hr were twice as active as the normal leaves.

It was considered preferable to present the CO_2 uptake measurements without correction for dark respiration, as it is likely that photorespiration was responsible for a larger part of the CO_2 evolved by the illuminated leaves. No attempt was made to measure photorespiration.

Photochemical Activities of Isolated Chloroplasts. Figs. 1, 2, and 3 show the influence of light intensity on the Hill reaction activities of chloroplasts isolated from normal and mutant leaves. With normal chloroplasts, the rate of reduction of $NADP^+$ was saturated at about 10,000 lux (Fig. 1), and this agrees reasonably well with the saturating intensities observed previously with spinach and normal barley chloroplasts (6). In contrast, the mutant chloroplasts required about 40,000 lux for saturation of $NADP^+$ reduction (Fig. 1). At saturating intensities, the mutant chloroplasts reduced $NADP^+$ at the rate of 185 μmoles/mg chl × hr, compared with 80 μmoles/mg chl × hr for normal chloroplasts. This 2.2 fold difference in activity for $NADP^+$ reduction on a chlorophyll basis closely duplicates the CO_2 uptake results.

When trichlorophenolindophenol (TCIP) was used as an electron acceptor however, the mutant chloroplasts were almost 5 times as active in dye reduction as the normal chloroplasts, if assayed at the very high light intensity of 200,000 lux (Fig. 2). The other feature of TCIP reduction by the mutant chloroplasts is that it was not possible to saturate the reaction even at an intensity of 200,000 lux. Normal chloroplasts were saturated at about 40,000 lux.

Ferricyanide reduction by the mutant chloroplasts was saturated at about 60,000 lux, compared with about 20,000 lux for normal chloroplasts (Fig. 3). The saturating activities were 850 and 280 μmoles ferricyanide reduced per mg chl per hr.

Electron Microscopy. Electron micrographs of chloroplasts from normal and mutant leaves are shown in Figs. 4 and 5. The grana are more distinct in the normal chloroplast and it appears that there are more lamellae per granum in the normal than in the mutant chloroplast. Table IV shows the results of the statistical analysis made on the lamellar counts from the 2 types of chloroplast. The number of grana per chloroplast and the number of single lamellae do not differ significantly, but the total number of lamellae per chloroplast and the number of lamellae per granum are highly significantly lower in the mutant. The number of large grana (with 8 or more lamellae) are also significantly lower in the mutant than in the normal.

Fig. 6 is an electron micrograph of a chloroplast from the yellow lethal mutant. It is characterized by an absence of lamellae pairing. Ribosomes are abundant within the chloroplast, from which it is concluded that the failure of the lethal mutant to synthesize normal grana or to form paired lamellae is not due to an absence of ribosomes.

Discussion

It is interesting to compare our chlorophyll-deficient pea mutant with the barley mutant lacking chlorophyll *b*, described previously (6, 11, 13), and the chlorophyll-deficient tobacco mutant (SU/Su) studied by Schmid and Gaffron (18, 19) and Homann and Schmid (14). The mutant barley leaves have about 70 % of the total chlorophyll of the leaves of normal barley plants (6) while the mutant tobacco has about 20 to 30 % of the chlorophyll of the normal tobacco (14). Our pea mutant leaves with 50 % of the chlorophyll of normal pea leaves are intermediate between the mutant barley and tobacco leaves in their

Table IV. *Statistical Analysis of Numbers of Lamellae and Grana in Normal and Mutant Chloroplasts*

Approximately 50 electron micrographs each of normal and mutant chloroplasts were taken at random and 10 each of normal and mutant chloroplasts were selected for distinctness and resolution of lamellae. The number of single lamellae, number of grana and number of lamellae per granum were counted and the results subjected to an analysis of variance.

No. of grana/ chloroplast	No. of lamellae/ chloroplast	Lamellae/ grana	No. of single lamellae/ chloroplast	No. of grana/ chloroplast with 8 or more lamellae
Normal				
40.3	270.5	6.52	8.8	12.7
Mutant				
38.6	179.1	179.	12.0	2.3
Difference				
1.7 N.S.[1]	91.4[2]	2.16[2]	—3.2 N.S.	10.4[2]

[1] N.S. Not significant.
[2] Highly significant, $P<0.001$.

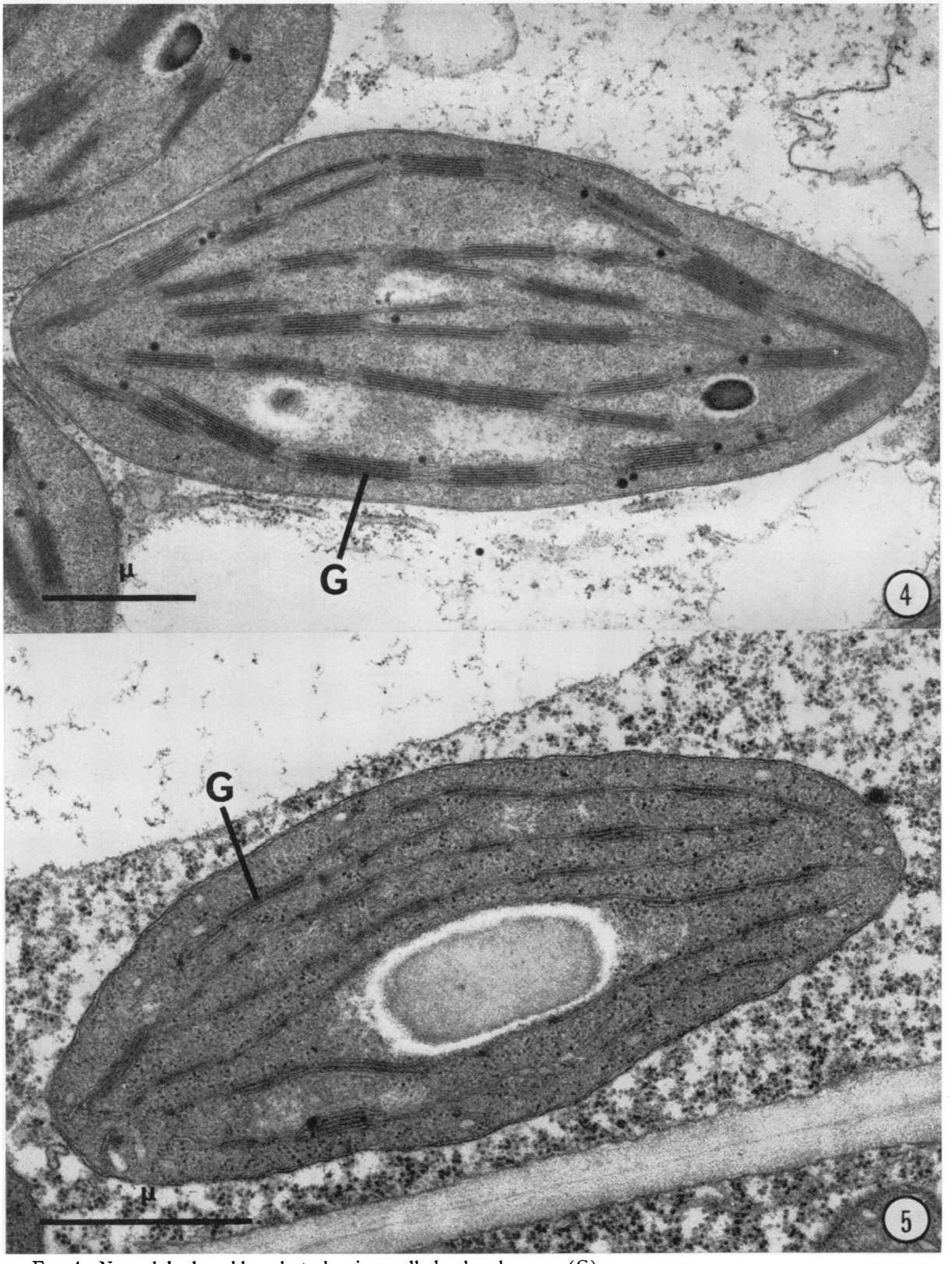

FIG. 4. Normal barley chloroplast showing well developed grana (G).
FIG. 5. Mutant barley chloroplast showing fewer lamellae per granum (G) than the normal.

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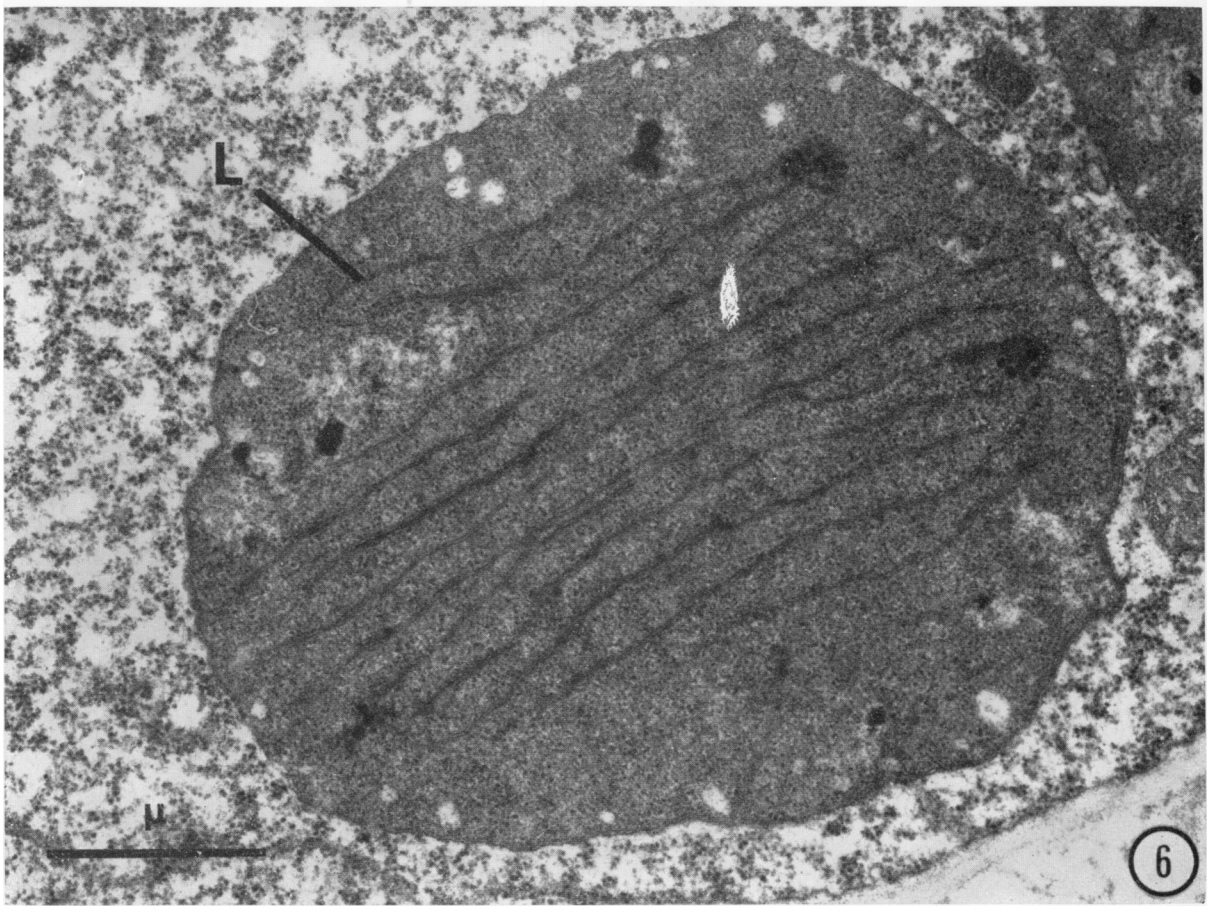

FIG. 6. Plastid from the lethal barley having no grana and a few single lamella (L).

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chlorophyll deficiency. Rates of CO_2 fixation by the various mutant plants at saturating light intensities, when expressed on a chlorophyll basis bear an inverse relationship to the chlorophyll content of the leaves. Thus, the normal barley plants gave a saturation rate of CO_2 fixation which was 70 % of the rate obtained with the mutant barley plants (13). The normal pea plant gave 46 % of the rate obtained with the mutant pea, and the normal tobacco about 24 % of the rate of the mutant tobacco.

Hill reaction activities at saturating intensities on isolated chloroplasts from barley and pea plants, with $NADP^+$ as oxidant, parallel the CO_2 uptake rates of intact leaves. The normal barley chloroplasts exhibited 75 % of the $NADP^+$ reducing activity of the mutant barley chloroplasts on a chlorophyll basis, (6), and the normal pea chloroplasts 43 % of the activity of the mutant pea. Normal barley chloroplasts reduced ferricyanide at 72 % of the rate observed with the mutant barley, while normal pea chloroplasts had 33 % of the ferricyanide-reducing activity of the mutant pea. The inverse relationship between chlorophyll content of the leaves and activity of isolated chloroplasts was obeyed for TCIP reduction by mutant and normal barley (6), but it did not hold so exactly for the pea chloroplasts, where the mutant showed considerably higher rates of TCIP reduction.

The results reported in this paper suggest that the photosynthetic units of the mutant pea chloroplasts contain about half the number of chlorophyll molecules of the photosynthetic units of normal chloroplasts. For the purposes of this discussion, we choose to define the photosynthetic unit as the minimum assembly of pigment molecules and associated electron carriers required for the transport of an electron from OH^- to $NADP^+$ (3), rather than as the number of chlorophyll molecules involved in the fixation of 1 molecule of CO_2 (10).

In a normal spinach (5) or pea chloroplast (unpublished observations) there is 1 molecule of cytochrome *f* per 400 chlorophyll molecules, from which it is concluded that the photosynthetic unit in a normal pea chloroplast contains about 400 chlorophyll molecules. The amount of P-700 supports this conclusion (15). The 400 chlorophyll molecules are thought to be divided about equally between the 2 pigment assemblies of photosystem 1 and 2 (3) leading to the concept of 2 units each containing approximately 200 light-harvesting chlorophyll molecules.

It is postulated from our present studies that the photosynthetic units (photosystems 1 + 2) of the mutant pea chloroplast contain about 200 chlorophyll molecules. Our recent finding, to be reported in a later publication, that the mutant pea chloroplasts contain twice the amount of cytochrome *f*, cytochromes b_6 and 559, of the normal pea chloroplasts, when expressed on a chlorophyll basis supports this conclusion.

The present studies do not allow a conclusion about the distribution of chlorophyll molecules between the photosystems of the mutant pea chloroplasts. Our earlier work (6, 7) with the barley mutant chloroplasts suggested that photosystem 2 of the mutant chloroplasts may contain less pigment molecules compared with photosystem 1. The mutant barley chloroplasts showed a lower photochemical efficiency at low light intensities (6) and lower quantum yields of fluorescence (7) than the normal barley chloroplasts. The Hill reaction curves (Figs. 1, 2, and 3) in the present paper do not show the cross-over points shown by the barley chloroplasts at 10 to 20 $\times$ 10^3 lux (6) but the ratio of the Hill activity of mutant and normal chloroplasts decreases as the light intensity is lowered. At the lower intensities the ratio is less than 2, which suggests that the quantum efficiency of the Hill reaction in the mutant chloroplasts is lower than that for the normal chloroplasts. Experiments are in progress to determine the quantum efficiencies of the mutant and normal pea chloroplasts in monochromatic light of low intensity. The higher saturation intensities observed with the mutant pea are considered to be due mainly to the lower chlorophyll content of the mutant's photosynthetic units, although a disproportion of chlorophyll between the photosystems would also be expected to increase the saturation intensities.

The structural differences between the normal and mutant pea chloroplasts particularly in respect to the number of grana per chloroplast are less than for the normal and mutant barley. The pea is more deficient in total chlorophyll than the barley, but the barley completely lacks chlorophyll *b*. The absence of chlorophyll *b* may impair grana formation or membrane pairing although the presence of grana in the barley mutant shows that chlorophyll *b* is not essential for grana formation.

The location of chlorophyll within the higher plant chloroplast has not been resolved. Fluorescence microscopy indicates that the red fluorescence of chlorophyll arises mainly from the grana (20), but this observation does not exclude chlorophyll from the intergrana lamellae, since the concentration of membranes in the grana is so much higher than in the intergrana regions. Some evidence is available which suggests that chlorophyll is uniformly distributed throughout the internal membrane system of the chloroplast (16). In their model of the chloroplast membrane Weier and Benson (21) postulate that chlorophyll is confined to the zones of membrane pairing (partitions).

Measurement of the total length of partitions on 3 mutant and 3 normal chloroplasts selected at random indicated that the mutant chloroplasts contained approximately 50 % of the partition length of the normal chloroplasts. This finding could be taken as evidence to support the view that chlorophyll is confined to the partitions, assuming a correlation between length and area of partition. Location of

chlorophyll in the partitions would suggest further that the area of partition occupied by a photosynthetic unit is determined by the number of chlorophylls in the unit and not by the carriers of the photosynthetic electron transport chain.

When measurements were made of the total length of partitions in electron micrographs of 3 mutant and 3 normal barley chloroplasts obtained previously (8) the total length of the partition in the mutant was only 35 % that of the normal barley chloroplast. Therefore, we are unable to conclude that there is a direct relation between chlorophyll content and length of partition.

Grana or membrane pairings appear to be completely absent from the lethal chloroplast. Chlorophyll is barely detectable in the leaves of the lethal plant and it seems likely that chlorophyll is essential for the formation of grana. The pea lethal chloroplasts resemble the chloroplasts in the yellow areas of the variegated tobacco materials studied by Homann and Schmid (14). These areas, however, did contain measurable amounts of chlorophyll *a* and chlorophyll *b*, and the chloroplasts, although low in Hill-reaction activity, were able to photoreduce $NADP^+$ if provided with an artificial electron donor (14).

Acknowledgments

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