Plant Physiol. (1970) 46, 692–698

Chloroplast Composition and Structure Differences in a Soybean Mutant[1]

Received for publication June 29, 1970

ROBERT W. KECK AND RICHARD A. DILLEY
The Charles F. Kettering Research Laboratory, Yellow Springs, Ohio 45387
C. FREEMAN ALLEN[2] AND SUSANNE BIGGS
Department of Chemistry, Pomona College, Claremont, California 91711

ABSTRACT

A nuclear mutation of *Glycine max* (soybean) segregates 1:2:1 in regard to chlorophyll content. The heterozygous (LG) leaf blade contains about one-half the pigment content of the wild type (DG) per gram fresh weight. A lethal yellow (LY) type contains about 1 to 2% of the DG leaf pigment values. The chlorophyll *a*/*b* ratio in the LG is about 5 compared to about 2 in the DG. Protein/leaf values are lower in the LG and LY types when compared to DG. The LG plastid lamellae contain more protein/chlorophyll, cytochromes/chlorophyll, and quinones/chlorophyll than the DG. P_{700}/chlorophyll values are similar in the DG and LG types.

The chlorophyll-depleted LG and LY types had less total acyl lipids per leaf weight when compared to the DG type. Similar amounts of sulfolipid and phosphatidyl glycerol per protein residue weight were found in the LG and DG plastids; however, the monogalactosyl and digalactosyl diglycerides were reduced in the LG paralleling the chlorophyll depletion.

Thin sections of leaf tissue show similar-sized LG and DG plastids but reduced grana formation in the LG. The LY has very few grana and very small grana compared to either DG or LG. The two characteristic particles revealed in higher plant chloroplasts by freeze-etching are about 15% smaller in the LG compared to the DG plants.

Mutations altering photosynthesis are found in many algae and higher plants (18). These mutations may be nuclear or nonnuclear and have been shown to affect pigments, electron transport chain components, and carbon cycle enzymes and structure (20).

The majority of algal and higher plant mutants which are depleted in chlorophyll content are lethal or grow very slowly (18). Several mutants have been described which at high light intensities photosynthesize at rates comparable to the wild type (8, 15, 16, 25). These chlorophyll-depleted mutants, as many other chlorophyll-depleted mutants, have limited or no lamellar stacking into grana.

Weber and Weiss (30) described a soybean nuclear mutation in which seeds collected from heterozygous plants segregate 1:2:1 in regard to chlorophyll content. The heterozygous plants are a light green (LG) and the homozygous plants are either normal dark green (DG) or a lethal yellow (LY). The yellow plants do not mature under normal physiological conditions but die when the colyledonary food reserves are depleted (about 9–14 days after germination).

Wolf (32) reported the pigment content of the aerial portions of these seedling types growing under 500 ft-c of fluorescent lighting at 24 C. The LG contained approximately one-half the pigment content of the DG, and the LY contained about 20% of the DG pigments. The rate of CO_2 fixed per leaf area of the LG was not significantly different from that of the DG while that of the LY was reduced. Carbon dioxide fixation rates in the three types, however, were light-saturated at the same intensity, unlike the above mentioned mutants.

Sun (27) reported reduced plastid size, limited grana stacking, and ultrastructural abnormalities in the later stages of LY plastid development.

This soybean mutation is unusual in the 1:2:1 segregation of the phenotypes and because the heterozygotes light-saturate at the same intensity as the normally pigmented plant.

This communication describes some pigment and nonpigment compositional differences and structural differences among the three phenotypes. A companion paper presents results of our studies concerning the comparative rates of electron and proton transport and photophosphorylation.

METHODS AND MATERIALS

Plant Culture Conditions. Soybean plants were grown hydroponically in a modified Hoagland's solution. Fluorescent lamps (Sylvania F96T12 wide spectrum and certified Spiralamp T-12 Slimline) were suspended about 1½ feet above the surface of the hydroponic solution yielding approximately 1500 ft-c. A time clock was employed to give a 12:12 light:dark cycle. The temperature ranged from approximately 15 C in the dark to approximately 32 C in the light.

Chloroplast and Lamellar Isolation. Excised leaf blades were ground in a Waring Blendor in an ice-cold solution containing 0.22 M sorbitol; 0.1 M KCl; 0.5 mM $MgCl_2$: 20 mM Tricine, pH 8.5; and 3 mM sodium ascorbate. The brei was forced through eight layers of cheesecloth, centrifuged at 1000*g* for 1 min to remove cell debris, and centrifuged again at 1000*g* for 10 min to pellet the chloroplast fraction.

The chloroplast fraction was washed twice in 50 mM KCl and 20 mM Tricine, pH 8.5, the pellet yielding the lamellar fraction.

[1] Contribution 399 from the Charles F. Kettering Research Laboratory, Yellow Spr ngs, Ohio 45387. Supported by National Institutes of Health Postdoctoral Fellowship FO2-GM-40707 to R. W. K. and in part by National Science Foundation Grant GB-8462 to R. A. D.

[2] Supported by Grant A1-04788 of the National Institutes of Health, United States Public Health Service.

Compositional Assays. Chlorophyll determinations were made according to Arnon (3), and protein was assayed by a modified Lowry (21) method. Extraction of lipids was carried out rapidly near 0 C to minimize enzymatic alteration of lipids. Leaf tissue was frozen in liquid nitrogen and pulverized to facilitate extraction. Chloroform-methanol (1:2, v/v) was used as an extractant in such quantity as to give one liquid phase including tissue water. Insoluble residue was removed on a sintered glass filter, and extraction was continued with additional small portions of the same solvent mixture until the residue was free of chlorophyll. Chloroform was used as the final extractant in the amount necessary to bring the combined extracts to a 1:1 (v/v) chloroform-methanol composition; 0.8 volume of cold water was then added to give a two-phase mixture. The upper (aqueous) layer was separated, washed with chloroform, and discarded. The combined chloroform-rich solutions were evaporated under nitrogen at room temperature or below and the residual lipid was taken up in chloroform-methanol (9:1, v/v) to a known volume. Aliquots were evaporated and weighed to determine total weight of lipid extracted.

The residue insoluble in these lipid solvents was dried at 100 C to constant weight which was used in calculating lipid-protein ratios.

The detailed description of the procedure for lipid analysis is in press elsewhere (1). Carefully measured aliquots of lipid solution containing about 700 μg of lipid extract were chromatographed in two dimensions on silicic acid thin layers, first in chloroform-methanol-water (65:25:4, v/v/v), and then in chloroform-methanol-isopropylamine-water (65:25:0.5:5, all by volume), after 10 min drying in a vacuum oven at 40 C. The lipids were made visible under ultraviolet light with a Rhodamine 6G-sodium hydroxide spary, and the appropriate areas of silicic acid were removed. The lipids, still on silicic acid, and a measured quantity of heptadecanoic acid internal standard were transesterified with 0.7 N sulfuric acid in absolute methanol at 70 C for 2 hr in a tightly capped vial. The cooled solution was diluted with an equal volume of water. Methyl esters were extracted with hexane and chromatographed on a 6-foot Reoplex 400 column at 198 C. A hydrogen flare ionization detector was used within its linear dynamic range. Molar quantities of methyl esters and thus the parent lipids were calculated from the area of methyl ester peaks relative to the internal standard. In cases where only total lipid content was determined the lipid extract was transesterified directly and a SE-30 column was used for quantitation.

Lipids were identified by comparison of chromatographic patterns with those of spinach leaves and other plant tissue which have nearly identical lipid composition. Further check on identity was made with spray reagents for amino groups, vicinal hydroxyls, and phosphorus. Furthermore, fatty acid compositions in the acyl groups of the various lipid classes had the unique patterns predicted (2).

Carotenoids were extracted from leaf blades with boiling 90% methanol. The extract was saponified with 10% KOH and partitioned with ethyl ether. Carotenoids were separated by thin layer chromatography, and quantities of individual carotenoids were determined spectrophotometrically from eluted spots (34).

Plastoquinones and α-tocopherol were extracted, purified, and assayed by procedures of Dilley (11) using thin layer chromatography to separate the quinones from other lipids obtained from three extractions of chloroplasts with 100% acetone. Tocopherol was purified by thin layer chromatography, converted to the quinone form by gold chloride oxidation, (12), and assayed by the spectrophotometric KBH_4 reduction method. An oxidized minus reduced extinction coefficient of 14.2 ΔA (at 260 nm) per μmole quinone per ml was used for calculating the α-tocopheryl-quinone content in a sample. A value of 14 ΔA at 255 nm was used for the calculation of the plastoquinone concentrations (13).

Cytochromes from washed lamellar fractions were assayed spectrophotometrically by addition of potassium ferricyanide and sodium dithionite to sample and reference cuvettes. Poor resolution of the cytochrome bands at room temperature required that we estimate total cytochromes from the difference spectrum.

For P_{700} assays, lamellar fractions were sonicated, and the light-dark difference spectrum at 700 nm was determined. The extinction coefficient used was 6×10^4 ΔA mole $^{-1}$ cm^{-2}.

Electron Microscopy. For thin sectioning, tissue slices were prefixed in 50 mM phosphate-buffered (pH 7.2) 2% glutaraldehyde, rinsed with buffer, postfixed in phosphate-buffered OsO_4, dehydrated in acetone, and imbedded in Epon-Araldite epoxy resin mixture (22). After sectioning, samples were post-stained with uranyl acetate.

For freeze-etching, the chloroplast fraction was pelleted in 20% glycerol, placed on copper discs, and rapidly frozen in Freon 22. Freeze-etching was accomplished according to Moor and Mühlethaler (23). Microscopy was done with a Philips EM 200 electron microscope.

RESULTS

Leaf blade protein and pigment determinations are shown in Table I. Only mature primary leaf blades were used. Four leaf blades of the DG and LG types yield about 1 g fresh weight whereas 15 LY leaf blades are needed for 1 g fresh weight. The amount of LG leaf blade proteins is reduced when compared to DG (Table I). The LY protein per g fresh weight is greater than either the LG or DG type. When compared on a per leaf basis, the LY protein content, like the pigment, is much reduced. LG leaf blades contain about one-half of the total amount of carotenoid and chlorophyll compared to the DG leaf blade. The LY blades were very much reduced in pigment when compared to either the LG or DG types.

The LG carotenoids, while reduced to one-half the DG carotenoids (Table I), show no significant differences in the percentage composition of individual carotenoids (Table II). The LY, however, contained only a trace of β-carotene.

The remainder of the compositional assays were determined with the use of the isolated washed lamellar fraction from mature primary leaf blades. Our isolation techniques did not yield a LY

Table I. *Leaf Blade Components*

Preweighed leaf blades were ground with a mortar and pestle in cold water (for protein assay) or in 80% cold acetone (for carotenoids and chlorophyll assays), thoroughly hand homogenized, and brought to a known volume. Aliquots were employed for spectrophotometric determinations.

	Protein	Carotenoid	Chlorophyll
	mg/g fresh wt	*μg/g fresh wt*	
DG	8.6 ± 0.5	359 ± 22	1663 ± 237
LG	4.9 ± 0.4	195 ± 2	673 ± 57
LY	9.5 ± 0.5	73 ± 12	45 ± 27

Table II. *Carotenoid Composition*

	DG	LG	LY
	% of total carotenoid weight		
β-Carotene	36.2	36.5	Trace
Lutein-zeaxanthin	33	32	61.6
Violaxanthin	19	22.5	17.8
Neoxanthin	11.8	9	20.6

lamellar fraction; therefore, the remainder of the compositional assays concern only LG and DG lamellar fractions.

The DG plastids, while containing twice the chlorophyll of the LG plastids, have a higher percentage of chlorophyll *b* than the LG (Table III). Figure 1 shows a portion of the visible spectrum of isolated washed lamellae of both the LG and DG types, indicating the presence of the chlorophyll *b* shoulder in the DG.

Other components of DG and LG lamellae, extensively washed in dilute salt, are shown in Table III. LG and DG lamellae have been compared on a protein and a chlorophyll basis. On a protein basis the LG lamellae have less chlorophyll, less acyl lipid, and less P_{700}. The LG has similar amounts of cytochrome/protein and plastoquinone/protein and twice as much α-tocopherol/protein when compared to DG.

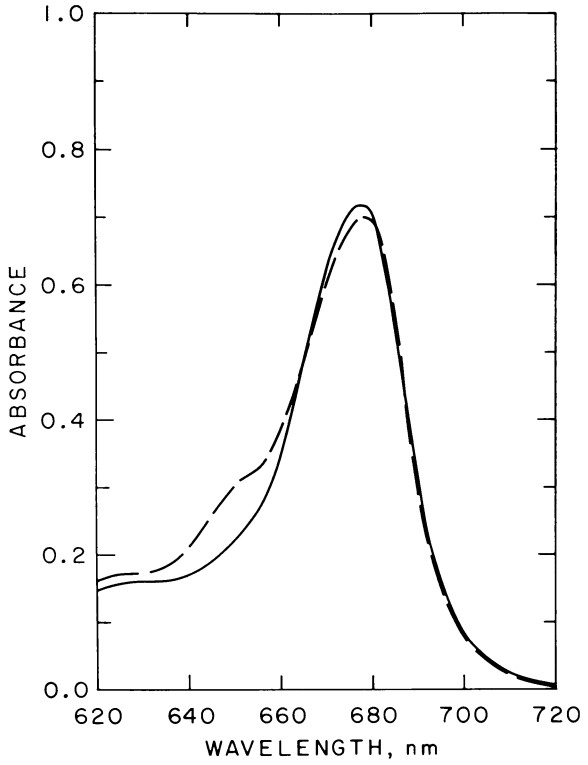

FIG. 1. An absorption spectrum for isolated plastids suspended in isolation medium. Dashed line: DG; solid line: LG.

On a chlorophyll basis the P_{700} values are quite similar. The plastoquinone/chlorophyll, cytochrome/chlorophyll, and protein/chlorophyll values in the LG are approximately two times that of the DG. The α-tocopherol/chlorophyll in the LG is about five times that of the DG. The total acyl lipid/chlorophyll is slightly higher in the LG when compared to DG. We have not yet determined the amount of individual cytochromes, plastocyanin, or ferredoxin in either LG or DG types.

The acyl lipid pattern of leaves from plants of the DG, the LG, and the LY were qualitatively identical in pattern and very similar to the pattern of spinach leaf used as a standard for comparison. However, the total acyl lipid content decreased markedly in the LG and LY (Table IV). Chloroplasts are rich in lipid which is localized in the lamellae, and a decrease is to be expected in the leaves with less developed chloroplasts. The acyl lipid content of chloroplasts from the LG was also lower than similar preparations from DG leaves. Again lipid patterns were qualitatively identical on thin layer chromatograms, but differences were apparent in quantitative determinations (Table V).

Monogalactosyl and digalactosyl diglycerides, phosphatidyl glycerol, and plant sulfolipid are the four lipids chraacteristic of the photosynthetic lamellae of higher plants (24), and these are the major lipids of the soybean chloroplasts. Small amounts of other lipids not characteristic of photosynthetic lamellae were present in the extracts but were not analyzed. However, the phosphatidyl ethanolamine found in small but varying amounts in each chloroplast preparation was determined and taken to be a measure of contamination of the preparation by other plant cell membranes, especially by those of mitochondria in which phosphatidyl ethanolamine makes up about a third of the total lipid. This lipid is absent or nearly so in chloroplasts (14). The low content of this lipid in the extracts indicates that the chloroplast preparations did not contain other material in amounts which would seriously alter the lipid ratios presented.

Free fatty acid is present in the lipid extracts to an extent of between 2 and 10% of the total acyl group content. This does represent a significant loss of lipid by enzymatic deacylation, probably during preparation of the chloroplasts, but it is not sufficient to alter the general conclusions on lipid composition patterns. Extensively washed chloroplast lamellae were also analyzed, but lipid degradation was so serious as to make the data useless. The distribution of fatty acids within each lipid class from lot II is given in Table VI. From the composition of the free fatty acid, it can be concluded that both galactolipids and anionic lipids have been degraded, and a higher percentage of galactolipids have been lost in the DG chloroplast preparation than in

Table III. *Isolated Lamellae Composition*

Assays runs in duplicate with eight different lamellar preparations with exception of acyl lipid.

	DG	LG	DG	LG
	per mg chlorophyll		*per mg protein*[1]	
Acyl lipid[2] (μmoles)	1.5	2.08	0.67	0.46
α-Tocopherol (mμmoles)	7.4 ± 0.6	35 ± 1	3.4 ± 0.3	8.0 ± 0.2
Plastoquinone *c* (mμmoles)	2.0 ± .5	10 ± 1	0.9 ± 0.2	2.2 ± 0.2
Plastoquinone *a* (mμmoles)	43 ± 3	104 ± 21	19.5 ± 0.7	23.7 ± 4.6
Cytochrome (mμmoles)	4.8 ± 0.5	10.5 ± 0.2	2.2 ± 0.2	2.3 ± 0.1
P_{700} (mμmoles)	1.4 ± 0.2	1.7 ± 0.3	0.66 ± .09	0.38 ± .07
Protein (mg)	2.2 ± 0.2	4.4 ± 0.1	...	...
Chlorophyll (mg)	...	...	0.46 ± 0.04	0.22 ± 0.05

	DG	LG
Chlorophyll *a*/*b* (μg/μg)	2.24	5.18

[1] Protein from lamellae washed three times with 0.05 M KCl and 0.02 M Tricine, pH 8.5.

[2] See Table V.

the mutant preparation. The absence of lysolipids in the extracts indicates that deacylation did not stop with loss of one acyl group.

Figure 2 reveals the relative plastid sizes of the three genotypes. Differences in grana stacking are evident in Figure 2. Table VII shows results of a quantification of grana stacking by the technique of Teichler-Zallen (28). The DG has fewer single lamellae and larger grana then the LG and LY types. The LY is almost entirely composed of single lamellae, seldom anastomosing.

Freeze-etch micrographs (Fig. 3) of the LG lamellae show groups of two types of particles, both of which are smaller than those of the DG. The larger particles of the LG averaged about 160 ± 30 A as compared to about 180 ± 30 A for the DG. Likewise the smaller particles are smaller in the LG (90 ± 10 A) when compared to those of the DG (100 ± 10 A). The LY lamellae, however, had only one type of particle (90 ± 20 A), which, unlike the smaller particles of the DG and LG lamellae, were widely separated.

Table IV. *Acyl Lipid Content of Soybean Leaves and Chloroplasts*

Leaf	Lipid Content[1]	Ratio of 16 to 18 Carbon Fatty Acids
DG leaf	59	0.61
LG leaf	41	0.76
LY leaf	23.5	1.26
DG chloroplast	212	0.21
LG chloroplast	172	0.41

[1] In total micromoles of diacyllipid per gram of dried residual leaf tissue after extraction of lipids.

Table V. *Acyl Lipid and Chlorophyll Content of Chloroplasts*

These data represent μmoles of acyl lipid and chlorophyll per gram of dried residual chloroplast tissue after extraction of lipids. Other minor lipids were present but not analyzed.

Lipid	DG		LG		Ratio:DG/LG		
	I[1]	II[1]	I	II	I	II	Avg
Monogalactosyl diglyceride	66	84	24	41	2.7	2.1	2.4
Digalactosyl diglyceride	52	104	15	35	3.5	3.0	3.2
Phosphatidyl glycerol	34	28.5	30	26	1.1	1.1	1.1
Plant sulfolipid	8	10	11	10	1.4	1.0	1.2
Phosphatidyl ethanolamine	3.6	2	1.9	5			
Fatty acid × ½	3.1	24.5	2.9	11.4			
Chlorophyll		165		59.3		2.8	

[1] Lot I was prepared in June, lot II in November.

Table VI. *Acyl Group Composition of Chloroplast Lipids*

Lipid	Chloroplast	Fatty Acid						
		16:0	16:1 (Δ^3 trans)	18:0	18:1	18:2	18:3	Others
		% of total[1]						
Monogalactosyl diglyceride	DG	3.4	0.5	0.5	0.3	2.1	93.0	
	LG	3.5		Tr	Tr	Tr	96.5	
Digalactosyl diglyceride	DG	16.3		1.8	Tr	1.3	80.4	
	LG	11.0		2.9		Tr	86.1	
Phosphatidyl glycerol	DG	26.6	38.9	7.8	12.8	4.7	5.6	3.4
	LG	34.4	30.6	5.2	7.5	9.8	12.5	
Sulfolipid	DG	45.7		7.3	2.5	4.3	40.0	
	LG	39.0		11.5	4.2	3.4	41.7	
Free fatty acid	DG	11.1	2.7	3.6	3.6	5.5	73.4	
	LG	30.2	5.0	12.6	11.7	9.6	30.7	

[1] Lipids of chloroplasts, lot II.

Table VII. *Quantification of Chloroplast Lamellar Structure*

	Percentage of Thylakoids Occurring Singly	Percentage of Grana with Five or more Thylakoids	Avg No. of Thylakoids per Grana	Range of Thylakoids per Grana
DG	9.0	26	4.98	23–2
LG	20.1	0	2.24	4–2
LY	68.2	0	2.15	3–2

DISCUSSION

While nuclear mutations inducing chlorophyll depletion in chloroplasts are common, the 1:2:1 segregation of the various phenotypes found in this soybean type is unusual. A pea mutant has been described, however, which has similar segregation (16). The soybean heterozygote (LG) photosynthetic rate light-saturates at the same intensity as the wild type (DG) (33). The pea mutant and other chlorophyll-depleted mutants (see Introduction) require higher light intensities for saturation when compared to the wild type.

The similar photosynthetic rates of the DG and LG plants (33) would tend to rule out the possibility of bulk chlorophyll or carotenoid content (or both) being the limiting factor of photosynthesis in this mutant. The LY, however, containing less than 2% of the DG chlorophyll concentration and about 16% of the DG carotenoid concentration, is probably photosynthetically limited by pigment concentration.

It is of interest to note the close correlation of the β-carotene concentration to that of chlorophyll concentration in the leaf blades of the three genotypes. The LG, for example, has about one-half the DG chlorophyll and carotenoid values (Table I). Since the percentage of β-carotene is the same in both LG and DG carotenoid components (Table II), the LG leaf blade contains about one-half the DG β-carotene value. The LY with very little chlorophyll has only a trace of β-carotene but has measurable amounts of the other carotenoids. These data corroborate the ideas of Blaauw-Jansen *et al.* (7) and the findings of Wallace and Habermann (29) in which chlorophyll content is related to β-carotene content and not total carotenoid content.

The same lipids are present in the wild type chloroplasts as in the mutant, and the acyl group compositions are essentially the same. There is, however, a striking difference in the glycolipid content (Table V). DG chloroplasts contain more than twice the molar quantity of the galactolipids (which are the most abundant lipids) per unit weight of dried tissue as do the LG chloroplasts, while the ionic lipids are nearly the same in both. The wild type chloroplasts also contain 2.8 times as much chlorophyll per unit weight of dry tissue as does the mutant. Therefore, the DG and LG have essentially the same monogalatosyl diglyceride/chlorophyll and digalactosyl diglyceride/chlorophyll ratios. However, the LG sulfolipid/chlorophyll and phosphatidyl glycerol/chlorophyll ratios are 3 to 4 times that of the DG.

The chlorophyll/P_{700} ratios were determined to show possible size differences in the LG and DG photosynthetic unit. Our data suggest similar chlorophyll/P_{700} ratios in both the LG and DG, indicating similar photosynthetic unit size. The light saturation data of Wolf (33) suggest also that the LG and DG photosynthetic units are similar. If the photosynthetic unit sizes are similar in the LG and DG types, the LG must have fewer photosynthetic units per leaf. The similar CO_2 fixation rates of Wolf (33) might

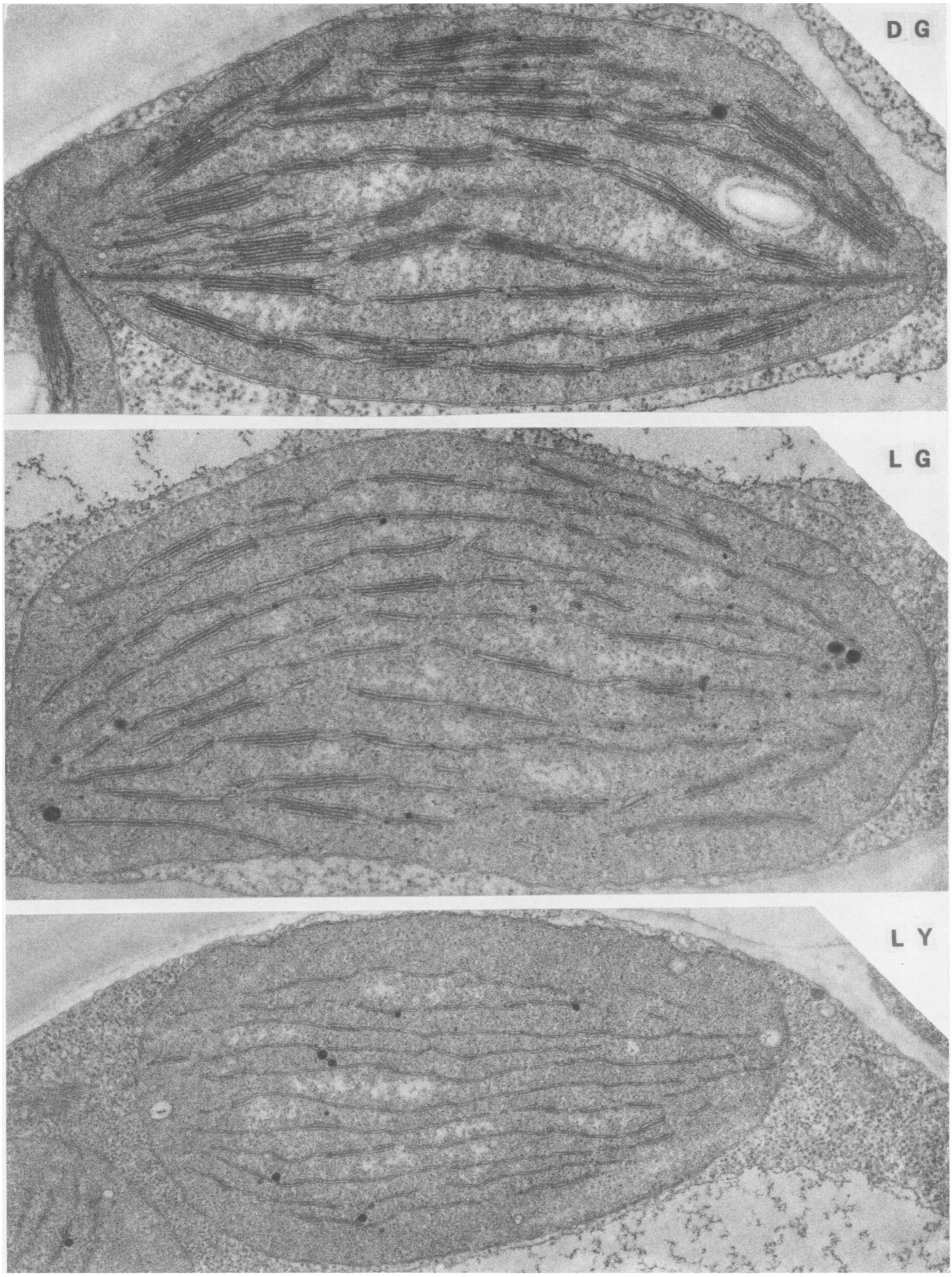

FIG. 2. Thin section of DG, LG, and LY leaf blade showing the relative chloroplast sizes and relative grana stacking in the three phenotypes. × 35,000.

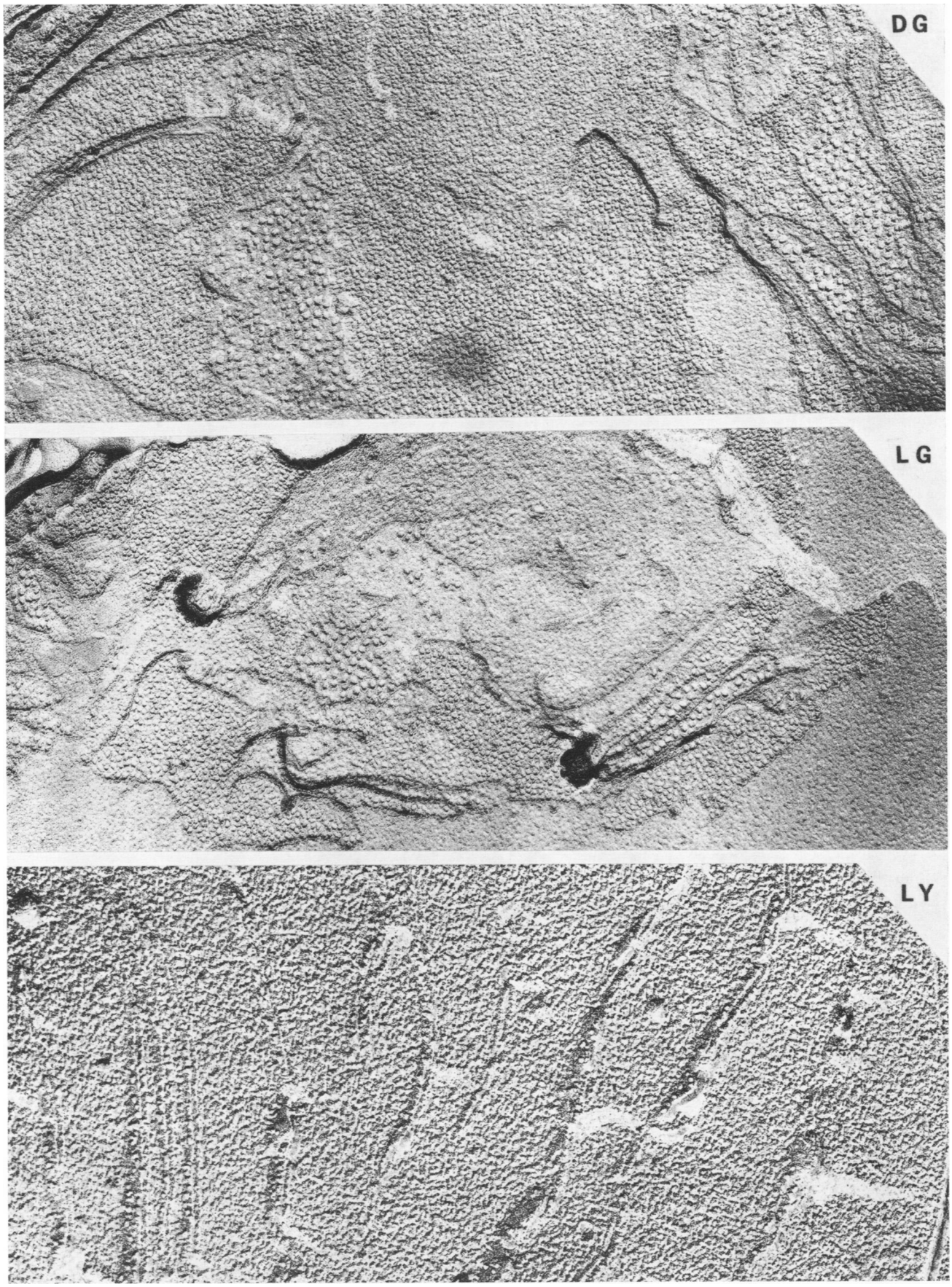

FIG. 3. Freeze-etch of the DG, LG, and LY lamellae revealing particle fields of large particles and small particles. × 64,000

then be explained by the DG plant not using all its photosynthetic units under saturating conditions, or the photosynthetic rate limitation imposed on the DG is relieved in the LG.

The concentration of cytochromes and quinones of the electron transport chain have been shown to be under nuclear control (20). Quinones have been shown to be present in chloroplast lamellae (10), involved in photosynthesis (5), and essential for cyclic phosphorylation (19). They were suggested by Weikard *et al.* (31) to be the acceptor of the electron flow from photosystem 2. Mutants of *Chlamydomonas reinhardi* (26) and *Scenedesmus obliquus* (6) have been shown to have low plastoquinone/chlorophyll values and are genetically blocked in photosystem 2 activity. The LG type containing more plastoquinone/chlorophyll may allow a greater photosynthetic rate/chlorophyll, as suggested in our companion paper (17).

While no direct photosynthetic function has been established for α-tocopherol, it is interesting that the α-tocopherol/chlorophyll ratio in the LG is higher than the α-tocopherol in the DG, paralleling that of the plastoquinone.

The number of plastids per leaf in the three genotypes are similar (unpublished observation); therefore, we feel the difference in leaf pigment content probably is due to differences in individual plastids rather than the number of plastids per leaf.

As in other chlorophyll-depleted mutants, the LG and LY types have limited or no lamellar stacking. While grana stacks are clearly evident in DG and LG, the majority of LY chloroplasts exhibit single lamellae with only occasional lamellar anastamosing. The LG has more single lamellae and smaller stacks of lamellae than the DG.

It appears unlikely that grana stacks are necessary for photosynthesis in *Chlamydomonas* (15). We are hesitant, therefore, to try to correlate, at this time, the significance of grana stacking and compositional differences in our soybean mutant.

We cannot, as yet, readily interpret the data concerning the reduced particle size in the LG revealed by freeze-etching. Arntzen *et al.* (4) equated the larger particles in a chloroplast membrane to a photosystem 2 marker and the smaller particles to a photosystem 1 marker. If the freeze fracture occurs along lipid-lipid interfaces as Branton and Park (9) have convincingly shown, it is possible that the lipoprotein particles embedded in the lipid matrix are different average sizes. These lipoprotein particles could well be smaller because of the reduced values of chlorophyll, carotenoid, and galactolipids in the LG plastid.

The companion paper (17) presents data concerning comparative photochemical and enzymatic activities of the wild type and LG mutant chloroplasts.

Acknowledgments—The authors appreciate the generous help of Dr. C. J. Arntzen in thin sectioning chloroplast samples for electron microscopy and likewise the aid of Dr. R. Crang in determining the number of plastids per leaf area by conventional histological techniques.

LITERATURE CITED

1. Allen, C. F. and P. Good. 1970. Photosynthesis and nitrogen fixation. *In:* A. San Pietro, ed., Methods in Enzymology, Vol. 23. Academic Press, New York. In press.
2. Allen, C. F., P. Good, H. F. Davis, P. Chisum, and S. D. Fowler. 1966. Methods for the separation of plant lipids and application to spinach leaf and chloroplast lamellae. J. Amer. Oil Chem. Soc. 43: 223–231.
3. Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 23: 1–15.
4. Arntzen, C. F., R. A. Dilley, and F. L. Crane. 1969. A comparison of chloroplast membrane surfaces visualized by freeze-etch and negative staining techniques; and ultrastructural characterization of membrane fractions obtained from digitonin-treated spinach chloroplasts. J. Cell Biol. 43: 16–31.
5. Bishop, N. I. 1959. The reactivity of naturally occurring quinone (Q-255) in photochemical reactions of isolated chloroplasts. Proc. Nat. Acad. Sci. U. S. A. 45: 1696–1702.
6. Bishop, N. I. 1969. Fluorescent and photochemical characteristics of system II mutants deficient in plastoquinone A. Biophys. J. 9: Soc. Abstr. A-118; TPM-H1.
7. Blaauw-Jansen, G., J. G. Komen, and J. B. Thomas. 1950. On the relation between the function of assimilatory pigments and the rate of photosynthesis in etiolated oat seedlings. Biochim. Biophys. Acta 5:179–185.
8. Boardman, N. K. and H. R. Highkin. 1966. Studies on a barley mutant lacking chlorophyll *b*. I. Photochemical activity of isolated chloroplasts. Biochim. Biophys. Acta 126: 189–199.
9. Branton, D. and R. B. Park. 1966. Subunits in chloroplast lamellae. J. Ultrastruct. Res. 19: 283–303.
10. Crane, F. L. 1959. Internal distribution of coenzyme Q in higher plants. Plant Physiol. 34: 128–131.
11. Dilley, R. A. 1964. Thin-layer chromatography of naturally occurring quinones and hydroquinones. Anal. Biochem. 7: 240–246.
12. Dilley, R. A. and F. L. Crane. 1963. A specific assay for tocopherols in plant tissue. Anal. Biochem. 5: 531–541.
13. Dilley, R. A. and F. L. Crane. 1964. Light-dependent conversions of endogenous α-tocopherylquinonone and plastoquinone-D in *Spinacia oleracea* chloroplasts. Plant Physiol. 39: 33–36.
14. Douce, R., T. Guillot-Salomon, C. Lance, and M. Signol. 1968. Étude comparée de la composition en phospholipides de mitochondries et de chloroplastes isolés de quelques tissues vegetaux. Bull. Soc. France Physiol. Veg. 14: 351–373.
15. Goodenough, U. W., J. J. Armstrong, and R. P. Levine. 1969. Photosynthesis properties of ac-31, a mutant strain of *Chlamydomonas reinhardi* devoid of chloroplast membrane stacking. Plant Physiol. 44: 1001–1012.
16. Highkin, H. R., N. K. Boardnan, and D. J. Goodchild. 1969. Photosynthetic studies on a pea-mutant deficient in chlorophyll. Plant. Physiol. 44: 1310–1320.
17. Keck, R. W., R. A. Dilley, and B. Ke. 1970. Photochemical characteristics in a soybean mutant. Plant Physiol. 46: 699–704.
18. Kirk, J. T. O. and R. A. E. Tilney-Bassett. 1967. The Plastids. Freeman and Company, London.
19. Krogmann, D. W. 1961. A requirement for plastoquinone in photosynthetic phosphorylation. Biochem. Biophys. Res. Commun. 4: 275–277.
20. Levine, R. P. 1969. Analysis of photosynthesis using mutant strains of algae and higher plants. Annu. Rev. Plant Physiol. 20: 523–540.
21. Lowry, O., N. Rosebrough, A. Farr, and R. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.
22. Mollenhauer, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. Stain Technol. 39: 111–114.
23. Moor, H. and K. Mühlethaler. 1963. Fine structure in frozen-etched yeast cells. J. Cell Biol. 17: 609–628.
24. Nichols, B. W. and A. T. James. 1968. Acyl lipids and fatty acids of photosynthetic tissue. *In:* L. Reinhold and Y. Liwschitz, eds., Progress in Phytochemistry, Vol. 1. Interscience, New York. pp. 1–48.
25. Schmid, G. H. and H. Gaffron. 1967. Light metabolism and chloroplast structure in chlorophyll-deficient tobacco mutants. J. Gen. Physiol. 50: 563–582.
26. Smillie, R. M. and R. P. Levine. 1963. The photosynthetic electron transport chain of *Chlamydomonas reinhardi*. II. Components of the triphosphopyridine nucleotide-reductive pathway in wild-type and mutant strains. J. Biol. Chem. 238: 4058–4062.
27. Sun, C. N. 1963. The effect of genetic factors on the submicroscopic structure of soybean chloroplasts. Cytologia 28: 257–263.
28. Teichler-Zallen, D. 1969. The effect of manganese on chloroplast structure of *Chlamydomonas reinhardi*. Plant Physiol. 44: 701–710.
29. Wallace, R. H. and H. H. Habermann. 1959. Genetic history and general comparisons of two albino mutations of *Helianthus annuus*. Amer. J. Bot. 46: 157–162.
30. Weber, C. R. and M. G. Weiss. 1959. Chlorophyll mutant in soybean provides teaching aid. J. Hered. 50: 53–54.
31. Weikard, J., A. Müller and H. T. Witt. 1963. The function of plastoquinone in the electron transport system of photosynthesis. Z. Naturforsch. 18: 139–141.
32. Wolf, F. T. 1963. The chloroplast pigments of certain soybean mutants. Bull Torrey Bot. Club 90: 139–143.
33. Wolf, F. T. 1965. Photosynthesis of certain soybean mutants. Bull. Torrey Bot. Club 92: 99–101.
34. Yamamoto, H. Y., C. O. Chichester, and T. O. M. Nakayama. 1962. Xanthophylls and the Hill reaction. Photochem. Photobiol. 1: 53–57.