Theor Appl Genet (1985) 69:279–284

Characterization of a cytoplasmically inherited yellow foliar mutant (*cyt-Y*$_3$) in soybean*

R. C. Shoemaker[1], A. M. Cody[2] and R. G. Palmer[3]

[1] Department of Genetics, [2] Bessey Electron Microscopy Facility, [3] United States Department of Agriculture, Agricultural Research Service, Departments of Agronomy and Genetics; Iowa State University, Ames, IA 50011, USA

Received April 20, 1984; Accepted May 29, 1984
Communicated by A. L. Kahler

Summary. Genetic analysis of a yellow foliar mutant in soybean (*Glycine max* L. Merr.) showed maternal inheritance of the mutant phenotype designated *cyt-Y*$_3$. The mutant was grown beside normal green sibs (*cyt-G*$_3$) under three different photosynthetic photon flux densities (PPFD), and samples were collected to determine pigment content and for electron microscopy analyses of plastid ultrastructure. The plastid ultrastructure of *cyt-Y*$_3$ appeared normal at low PPFD and the carotenoid level of *cyt-Y*$_3$ was also normal, but the chlorophyll content was only approximately one-third that of *cyt-G*$_3$. Under medium and high PPFD, *cyt-Y*$_3$ plastids lacked a structured thylakoid, and total chlorophyll content was only 28% and 1% of normal, respectively; the carotenoid levels of *cyt-Y*$_3$ also dropped to 33% and 2% of normal, respectively. These data indicate that the effect of high PPFD on *cyt-Y*$_3$ might result from a deficiency in a plastid membrane protein. The resulting changes in membrane configuration could then interfere with the accumulation or stabilization of chlorophylls and carotenoids, thereby resulting in the subsequent photooxidation of both at medium and high PPFD. This mutant could be useful in the study of thylakoid biosynthesis and pigment stabilization, or could provide a source of conditionally identifiable plastids for organelle segregation studies.

Key words: Nuclear-cytoplasmic interaction – Chlorophyll – *Glycine max* L. – Maternal inheritance – Plastid ultrastructure

* This is a joint contribution of North Central Region, USDA-ARS., and Journal Paper No. J-11124 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA 50011, Project 2471. Mention of a trademark of proprietary product by Iowa State University or the USDA does not imply its approval to the exclusion of other products that may also be suitable

Introduction

The occurrence of plant chimeras in nature is a common phenomenon. Chimeras are a direct result of the segregation of normal and mutant organelles into genotypically and phenotypically distinct vegetative sectors (Neilson-Jones 1969). Entirely yellow or entirely green branches may develop, depending upon the assortment of organelles in meristematic regions. When a floral part develops in a mutant sector, the mutant phenotype will be transmitted through the maternal gamete. Occasionally, normal organelles will be transmitted from mutant sectors. This may result in subsequent expression of normal sectors in an otherwise mutant progeny. It is not known whether the expression of normal tissue is the result of an 'absolute' assortment of normal organelles or the result of a threshold effect.

Interactions of normal nuclear and normal cytoplasmic factors may result in pigment deficiencies (Stubbe 1964; Schotz 1970). However, most pigment deficiencies are the direct result of either nuclear or cytoplasmic mutations.

In soybean (*Glycine max* L.), at least 14 different nuclear mutations for chlorophyll deficiency currently exist (Bernard and Weiss 1973). Two of these, y_{18}^{m} and y_{11}, have been analyzed for plastid ultrastructural aberrations. The thylakoid systems in y_{18}^{m} were shown to be disrupted, resulting in only isolated grana (Palmer et al. 1979). The thylakoid system in y_{11} was shown to be restricted to long parallel lamellae that exhibited very few overlapping thylakoids (Crang and Noble 1974). The aberrant ultrastructure noted in plastids of y_{11} seems to be common among chlorophyll-deficient mutants. Similar patterns have been reported in maize (Bachmann et al. 1973), oats (Muruyama 1961), tobacco (Schmid and Gaffron 1967), and barley (MacLachlan and Zolek 1964). Organization of the thylakoid system appears to be dependent upon such environmental factors as temperature (Ballantine and Forde 1970) and light intensity (Ballantine and Forde 1970; Meier and Lichtenthaler 1981).

A cytoplasmically inherited green seed embryo trait, *cyt-G*$_1$, was reported in soybean by Terao (1918). The first cyto-

plasmically inherited yellow foliar mutant in soybean was reported by Palmer and Mascia (1980). This mutant was designated *cyt-*Y_2 and was characterized by reduced chlorophyll content with progressive greening under both field and growth chamber conditions and by normal plastid ultrastructural development. We report here the characterization of a second cytoplasmically inherited yellow foliar mutant in soybean, *cyt-*Y_3.

The objectives of our study were to determine the mode of inheritance of the mutant phenotype, and to characterize the plastid ultrastructure and pigment content of mutant plants grown under a wide range of photosynthetic photon flux densities.

Materials and methods

Origin and observations

In 1972, two seeds from a chimeric soybean plant were obtained from R. C. Clark, USDA, ARS, Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. The original cultivar was unknown. These seeds were grown in the field during the summer of 1973 and produced chimeric plants. Seeds from these chimeras were planted in the field in the summer of 1977, and the resulting plants were either chimeras, normal green, or lethal yellow. This mutant yellow line was assigned a laboratory Genetic Type Collection Number (T278), and the gene symbol *cyt-*Y_3 was assigned by the Soybean Genetics Committee. The normal green sibling line was designated *cyt-*G_3.

Genetic studies

Cross-pollinations were made using standard soybean technique (Paschal 1976). Floral parts from predominantly yellow branches of chimeras (*cyt-*Y_3) were used to maximize the transmission of the mutant trait. The cultivar 'Illini' was used as the female parent. 'Illini' is homozygous for w_1/w_1 (white flower), and *cyt-*Y_3 is homozygous for W_1/W_1 (purple flower). Segregation of plants for purple and white flower color in the F_2 generation provided evidence that F_1 hybrids were produced.

Crosses also were made by using yellow branches of the *cyt-*Y_3 chimera as female parents and 'PI 189866' and 'Minsoy' as male parents. Mutant line *cyt-*Y_3 is homozygous for blunt pubescence tip (*pb/pb*). 'PI 189866' and 'Minsoy' are homozygous for the sharp pubescence tip (Pb/Pb). 'PI 189866' also is homozygous for a chromosome translocation (Sadanaga and Newhouse 1982) and *cyt-*Y_3 is homozygous for the normal chromosome arrangement. In both crosses the presence of sharp pubescence tips or 50% pollen sterility (translocation heterozygote) in the F_1 plants confirmed the hybrid origin of the F_1.

Reciprocal crosses also were made between *cyt-*Y_3 chimera and k_2 (Genetic Type T239). Segregation of the tan-saddle pattern seed coat trait (k_2) among the F_2 provided evidence that F_1 hybrids were successfully produced.

Growth conditions

Preliminary results indicated that yellow *cyt-*Y_3 mutant plants survived better at lower light intensities. Consequently, we were interested in pigment accumulations and plastid ultrastructure from plants grown in various photosynthetic photon flux densities (PPFD).

Seed of *cyt-*G_3 (green progeny from chimeras) and *cyt-*Y_3 (yellow progeny from chimeras) were germinated according to the procedure of Palmer and Heer (1973). After 5 days, the germinated seed were transferred into pots containing a 3 : 1 : 1 mixture of loam, sand, and peat and placed in environmental chambers.

Three different environmental chambers were used to provide PPFD's of 90, 600, and 2,050 microeinsteins M^{-2} s. Illumination was provided for 14 h per day. Daytime and nighttime temperatures were set at 24 ± 1 °C and 21 ± 1 °C, respectively.

Seedlings were grown 16 to 18 days before leaflet samples were taken for pigment determinations and for electron microscopy studies.

Pigment determinations

Primary trifoliolates were collected from 21- to 23-day-old seedlings, placed immediately on ice, and homogenized at 4 °C in 85% acetone using a chilled mortar and pestle. The homogenate was centrifuged at 5,000 rpm at 4 °C for 10 min, the supernatant filtered through a Whatman No. 1 filter. Absorbances were determined at 452 nm, 644 nm, and 663 nm and pigment concentrations were determined using the equations of Röbbelen (1957):

chl a (mg/l) = 10.3 ($^{0D}663$) − 0.918 ($^{0D}644$)
chl b (mg/l) = 19.7 ($^{0D}644$) − 3.87 ($^{0D}663$)
carotenoids (mg/l) = 4.75 ($^{0D}452$) − (chl a + chl b) (0.226).

Electron microscopy

Concurrent with sample collection for pigment concentration determinations, leaf punches were taken for electron microscopy comparisons. Leaf punches from primary trifoliolates were fixed in 2% paraformaldehyde–2.5% glutaraldehyde in phosphate buffer (pH 7.24) for 2 h at 4 °C (Glauert 1975). Specimens were rinsed in a cold 1 : 1 solution of buffer and distilled water and placed in glutaraldehyde in phosphate buffer for 24 h at 4 °C. They were then rinsed in buffer and distilled water (1 : 1), post-fixed with 2% OsO_4, dehydrated in a graded acetone series, and infiltrated with Medcast. Sections were cut with a diamond knife and stained with lead citrate and uranyl acetate (Reynolds 1963) and viewed on a Hitachi HU-11C transmission electron microscope.

Results

Genetic studies

Results of reciprocal crosses made to determine the mode of inheritance of the *cyt-*Y_3 phenotype are shown in Table 1. In the crosses 'Illini' × 'Chimera', 'Chimera' × 'Minsoy', T239 × 'Chimera', and 'Chimera' × T239, single gene markers were used to verify the hybrid origin of the progeny. In the cross 'Chimera' × 'PI 189866', a single gene marker and semi-sterility resulting from chromosome interchange heterozygosity were used to verify successful hybridization.

The absence of segregation for the mutant chimera phenotype in the F_2 generation of the crosses 'Illini' × 'Chimera' and T239 × 'Chimera' suggests that the mutant trait is not transmitted through the pollen and most likely is a cytoplasmically carried trait. The

Table 1. Observed numbers of progeny in F_1 and F_2 generations of soybean crosses between *cyt-Y*$_3$ Chimera and 'Illini', 'Minsoy', 'PI 189866', and T239

Parents		F_1 generation	F_2 generation
Female	Male		
'Illini' (w_1/w_1)	× Chimera (W_1/W_1)	20 *cyt-G*$_3$	all *cyt-G*$_3$ seg. W_1/W_1
Chimera (pb/pb)	× 'PI 189866' (Pb/Pb)	5 *cyt-Y*$_3$ sharp tips 50% pollen sterility	
Chimera (pb/pb)	× 'Minsoy' (Pb/Pb)	4 *cyt-Y*$_3$ sharp tips 1 Chimera[a] sharp tips	16 *cyt-Y*$_3$ 4 Chimera
T239 (k_2k_2)	× Chimera	2 *cyt-G*$_3$	70 *cyt-G*$_3$, nonsaddled seed 29 *cyt-G*$_3$, saddled seed
Chimera	× T239 (k_2k_2)	4 *cyt-G*$_3$ 10 cyt-Y$_3$ 5 Chimera[1]	154 *cyt-G*$_3$, nonsaddled seed 47 *cyt-G*$_3$, saddled seed 54 *cyt-Y*$_3$, no seed set (died) 89 *cyt-G*$_3$ 1 *cyt-Y*$_3$ 30 Chimera

[a] Progeny of Chimeras were scored for segregation of the mutant trait at the primary trifoliate stage

Table 2. Pigment concentrations (mg/g fresh wt) in mutant *cyt-Y*$_3$ and normal *cyt-G*$_3$ soybean lines grown under PPFD's of 90, 600, or 2,050 microeinsteins M^{-2} s

PPFD 90			
Pigment	*cyt-G*$_3$	*cyt-Y*$_3$	*cyt-Y*$_3$ as % of *cyt-G*$_3$
Carotenoids	0.093±0.007	0.093±0.010	100%
Total chlorophyll	0.664±0.047	0.226±0.007	34%
a/b ratio	2.272±0.110	2.535±0.060	
	PPFD 600		
Carotenoids	0.227±0.027	0.076±0.007	33%
Total chlorophyll	0.774±0.009	0.216±0.007	28%
a/b ratio	2.215±0.060	2.428±0.090	
	PPFD 2,050		
Carotenoids	0.266±0.024	0.004±0.0004	2%
Total chlorophyll	1.048±0.048	0.009±0.001	1%
a/b ratio	2.435±0.020	0.953±0.360	

maternal inheritance of the yellow phenotype in crosses with 'PI 189866' and 'Minsoy', and in the cross 'Chimera' × T239 is corroboratory.

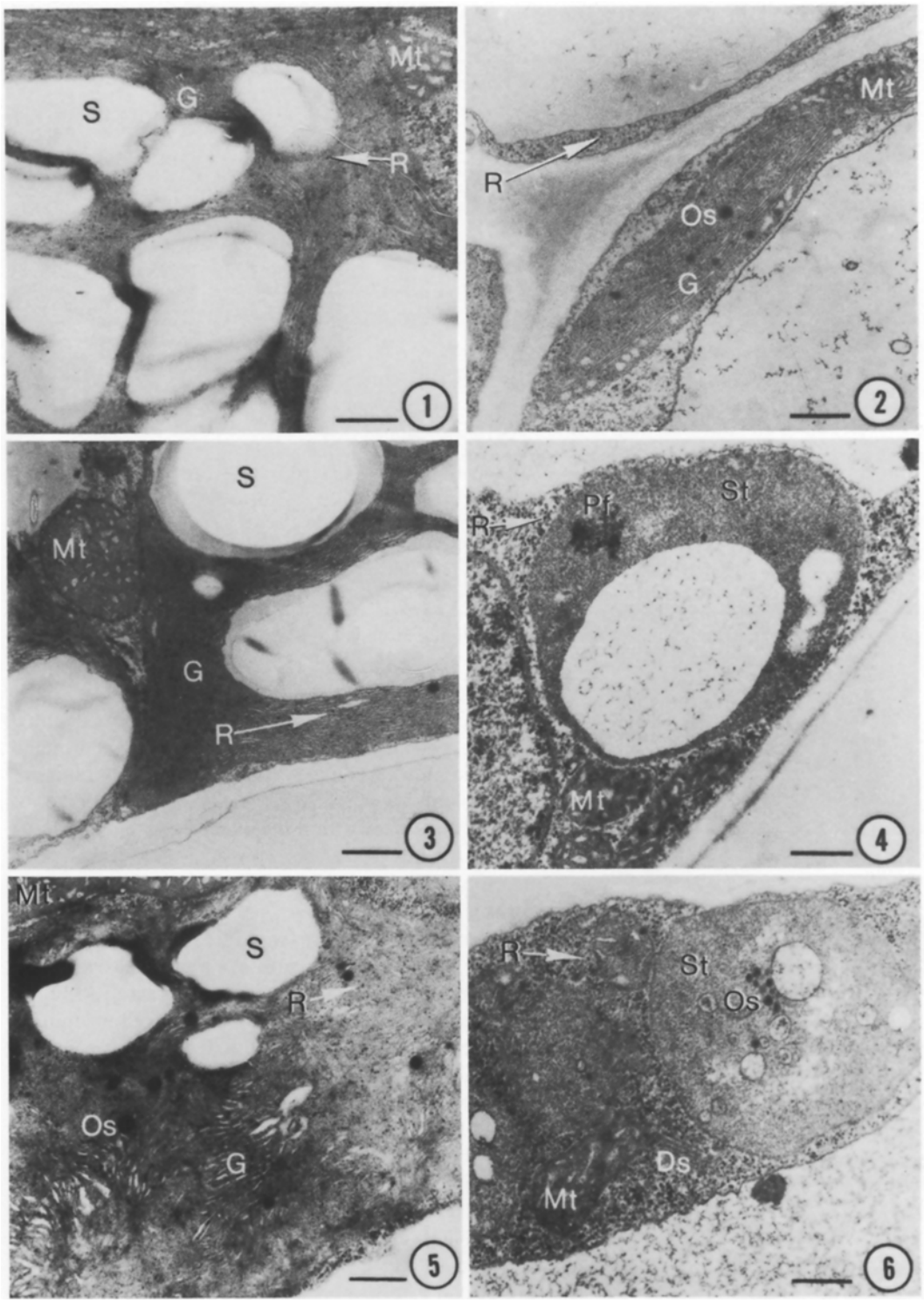
Pigment determinations

Results of pigment determinations from the three different PPFD regimes are shown in Table 2.

Yellow plants and green plants developed equal amounts of carotenoids at PPFD 90, and chlorophyll a/b ratios also were very similar. The a/b ratio for yellow plants was slightly greater than for green plants, but the ratios were within the expected range for young soybean plants. Total chlorophyll in the yellow plants was approximately one-third the level found in green plants.

A concomitant increase in carotenoid level was noted in green plants when PPFD was increased to 600. Yellow mutants did not respond to increased PPFD in this manner. Carotenoid level of the yellow mutant decreased slightly. Chlorophyll a/b ratios were similar between green plants and yellow plants, and the a/b ratio for yellow plants was slightly greater than for green plants. Total chlorophyll in yellow plants was slightly less than one-third the level in green plants.

Green plants produced more than 1 mg chlorophyll/g fresh weight while chlorophyll production of yellow plants dropped precipitously when PPFD was increased to 2,050, approximately the intensity of full sunlight on a horizontal surface. The carotenoid level of yellow plants also was at a barely perceptible level, (2% of the level found in green plants) and the chlorophyll a/b ratio of yellow plants dropped to approximately 1. At this high PPFD, yellow plants produce only about 1% of the total chlorophyll produced by normal green plants. As might be expected from these data, high light intensities are lethal to the yellow mutant.



Figs. 1–6. Photomicrographs of plastids from *cyt-G*$_3$ and *cyt-Y*$_3$ seedlings grown under three different PPFD (*G* = grana, *Ds* = dictyosome, *Mt* = mitochondrium, *Os* = plastoglobuli, *Pf* = phytoferritin molecules, *R* = ribosomes, *S* = starch, *St* = stroma). Bar scales represent 0.5 μm. **1, 3, and 5.** Plastids of *cyt-G*$_3$ seedlings grown under PPFD 90, 600, and 2,050 microeinsteins M^{-2} s, respectively. **2, 4, and 6.** Plastids of *cyt-Y*$_3$ seedlings grown under PPFD 90, 600, and 2,050 microeinsteins M^{-2}, respectively

Electron microscopy analysis

Samples were collected for electron microscopy analyses concurrently with samples collected for pigment determinations. The results of the electron microscopy comparisons are shown in Figs. 1–6.

The plastids of yellow plants appeared normal in most respects when compared with plastids of normal green sibs at PPFD 90. Plastids of the mutant, however, were smaller than plastids of the normal greens, and mutant plastids showed a conspicuous absence of starch. Both green plants and yellow plants exhibited a well-defined plastid thylakoid system with long parallel lamellae and grana stacking, normal-appearing ribosome distribution in the plastid stroma as well as in the cytoplasm, and normal-appearing mitochondria.

When PPFD was increased to 600, the grana stacking in plastids of green plants was very well organized, but completely absent in plastids of yellow plants. Plastids of yellow plants again contained no visible starch deposits. The thylakoid system in plastids of green plants showed a disorganization and a widening of the separations between the granal discs at PPFD 2,050. Ribosomes were clearly visible and well distributed in the cytoplasm and in the plastid stroma of green plants at PPFD's 600 and 2,050. In yellow plants, ribosomes were clearly visible and well distributed in the cytoplasm but were seen only infrequently in the plastid stroma.

Discussion

Much classical genetic information has been the direct result of mutation. Before a genetic function can be determined unambiguously, a concomitant genetic malfunction must be recognized. Mutations in organelles are difficult to obtain because of the multiple copies of the organelles, the polyploid nature of the organelle, and the competitive disadvantage of most mutations. Therefore, obtaining and characterizing a new cytoplasmic mutant may provide valuable material for the subsequent study of organelle genome functions and nuclear-cytoplasmic interactions.

The presence of green and chimeric plants in the F_1 generation from a cross involving a predominantly yellow branch of a chimera, as female, is not discordant with the uniparental inheritance of the *cyt-Y*$_3$ phenotype. It is expected that 'normal' organelles are present in some cells of a mutant sector of a chimera. If the cell containing both mutant and normal organelles is located in reproductive tissue, the normal phenotype may be transmitted. This transmission could result in normal green plants or more chimeras.

A paucity of grana stacking in a chlorophyll-deficient mutant is a fairly common phenomenon, and, because of this the *cyt-Y*$_3$ phenotype is not unique. An underdeveloped ultrastructure has been shown to be typical of plastids that are lacking in chlorophyll and carotenoids (Shumway and Weier 1967; Murakami 1962). Therefore, the absence of a structured thylakoid system in the mutant plastids at PPFD's where chlorophyll and carotenoid levels were declining is expected.

Carotenoids have been implicated in the protection of chlorophyll from photooxidation (Robertson et al. 1966; Anderson and Robertson 1960). Therefore, loss of carotenoids could account for some reduction in chlorophyll content at higher PPFD. However, the reduced chlorophyll content in *cyt-Y*$_3$, at PPFD 90, accompanied by a normal carotenoid level at the same PPFD precludes the suggestion that the reduction in chlorophyll at higher PPFD's is due totally to carotenoid loss.

More likely, the *cyt-Y*$_3$ phenotype is the result of a mutation in a plastid encoded gene for a membrane protein. Possibly, this mutation has occurred in a gene coding for a plastid membrane protein essential for normal grana formation. The resulting changes in membrane configuration could conceivably interfere with the accumulation and/or stabilization of both chlorophylls and carotenoids. Therefore, medium and high PPFD's could result in photodestruction of both types of pigments. The reduction in chlorophyll content at PPFD 90 suggests that chlorophyll is more sensitive to destabilization than are the carotenoids.

Ribosomes play an integral part in the translational apparatus of a plastid. In maize, iojap (*ij*) induces abnormal plastid differentiation through a reduction in chloroplast ribosome numbers (Walbot and Coe 1979). The presence of a normal number of ribosomes in the stroma of *cyt-Y*$_3$ plastids at a low PPFD would preclude the argument that abnormal plastid development might be due to a defect in chloroplast ribosomes, which would hinder protein synthesis and subsequent thylakoid development. The paucity of ribosomes in the stroma of mutant plastids at medium and high PPFD may be a secondary effect of the disruption of the plastid internal systems.

On the basis of our analysis of this mutant, we conclude that the mutant phenotype is inherited maternally and is probably the result of photooxidation of chlorophylls, coupled with an inability to develop a structured thylakoid system under moderate to high PPFD.

Though this mutant probably has limited value to classical soybean geneticists, it may be of interest to plant physiologists and somatic cell geneticists. The responsiveness of the mutant to various PPFD may provide membrane physiologists with a vehicle by which to study the biosynthesis of the thylakoid and the stabilization of chlorophylls. The distinguishing characteristics of mutant plastids at medium and high PPFD, but not at low PPFD, may provide an interesting basis for cybrid production and organelle segregation studies.

References

Anderson IC, Robertson DS (1960) Role of carotenoids in protecting chlorophyll from photodestruction. Plant Physiol 35:531–534

Bachmann MD, Robertson DS, Bowen CC, Anderson IC (1973) Chloroplast ultrastructure in pigment deficient mutants of *Zea mays* under reduced light. J Ultrastruct Res 45: 384–406

Ballantine JEM, Forde BJ (1970) The effect of light intensity and temperature on plant growth and chloroplast ultrastructure in soybean. Am J Bot 57:1150–1159

Bernard RL, Weiss MG (1973) Qualitative genetics. In: Caldwell BE (ed) Soybeans: improvement, production, and uses. Am Soc Agronomy, Madison Wis, pp 117–154

Crang RE, Noble RD (1974) Ultrastructural and physiological differences in soybeans with genetically altered levels of photosynthetic pigments. Am J Bot 61:903–908

Glauert AM (1975) Fixation, dehydration, and embedding of biological specimens. In: Glauert AM (ed) Practical methods in electron microscopy. North Holland, New York, pp 5–72

MacLachlan S, Zolek S (1964) Plastid structure, chloroplast concentration, and free amino acid composition of a chlorophyll mutant of barley. Can J Bot 41:1053–1062

Meier D, Lichtenthaler HK (1981) Ultrastructural development of chloroplasts in radish seedlings grown at high- and low-light conditions and in the presence of the herbicide bentazon. Protoplasma 107:195–207

Murakami S (1962) Electron microscope studies on plastid development in variegated leaves of *Liriope platyphylla* f. Variegata hort. 2. The albicate plastid. Cytologia 27: 140–150

Muruyama K (1961) Electron microscope observations on the development of chloroplasts of *Avena* and chlorophyll deficient mutants. Cytologia 26:105–115

Neilson-Jones W (1969) Plant chimeras. Methuen and Co, London

Palmer RG, Heer H (1973) A root tip squash technique for soybean chromosomes. Crop Sci 13:389–391

Palmer RG, Mascia PN (1980) Genetics and ultrastructure of a cytoplasmically inherited yellow mutant in soybeans. Genetics 95:985–1000

Palmer RG, Sheridan MA, Tabatabai MA (1979) Effects of genotype, temperature, and illuminance on chloroplast ultrastructure of a chlorophyll mutant in soybeans. Cytologia 44:881–891

Paschal EH II (1976) Crossing soybeans. In: Hill LD (ed) World soybean research. Interstate Printers and Publishers, Danville ILL, pp 266–267

Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17:208–212

Röbbelen F (1957) Untersuchungen an strahleninduzierten Blattfarbmutanten von *Arabidopsis thaliana* (L.) Heynh Z Indukt Abst Vererbungsl 88:189–252

Robertson DS, Bachmann MD, Anderson IC (1966) Role of carotenoids in protecting chlorophyll from photodestruction. 2. Studies on the effect of four modifiers of the albino cl_1 mutant of maize. Photochem Photobiol 5:797–805

Sadanaga K, Newhouse K (1982) Identifying translocations in soybeans. Soybean Genet Newslett 9:129–130

Schmid G, Gaffron H (1967) Light metabolism and chloroplast structure in chlorophyll deficient tobacco mutants. J Gen Physiol 50:563–582

Schotz F (1970) Effects of disharmony between genome and plastome on the differentiation of the thylakoid system in Oenothera. In: Miller PL (ed) Control of organelle development. Soc Exp Biol Symp 24:39–54

Shumway WL, Weier TE (1967) The chloroplast structure of *iojap* maize. Am J Bot 54:773–780

Stubbe W (1964) The role of plastome in evolution of genus *Oenothera*. Genetica 35:28–33

Terao H (1918) Maternal inheritance in the soybean. Am Nat 52:51–56

Walbot V, Coe EH Jr (1979) The nuclear gene *iojap* conditions a programmed change to ribosome-less plastids in *Zea mays*. Proc Natl Acad Sci USA 76:2760–2764