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Genetic Control of Chloroplast Pigment Development in Soybeans as a Function of Leaf and Plant Maturity

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ABSTRACT

Changes in the major chloroplast pigments of pigment-deficient genotypes of soybeans were studied as a function of leaf age and plant age. The trends of pigment development are plotted as a function of relative leaf age at two periods of plant development. Comparisons are made between aging leaves from different nodes and leaves of different ages from the same node. In addition, trends of pigment development are expressed as a ratio of pigment-deficient/normal genotypes for five different genotypes and seven sampling periods. Those genotypes that exhibit a lag in production of pigments during leaf development show a similar lag in overall plant pigment development.

Genetic control of chloroplast development in higher plants can be effectively studied by use of pigment-deficient mutant genotypes which commonly occur in most plant species. These mutants run the full range of pigment composition but are usually deficient in Chl *a*, Chl *b*, or both carotenoids and Chl. Early descriptions of typical mutants occur in the literature for maize (9), barley (10), and soybeans (17). These early reports used spectroscopic methods and crude column separation to give information on Chl *a* and *b*, and ratios of carotenoids to Chl. Improved techniques of analysis, such as the two-dimensional paper chromatographic study of pigments in peanut mutants by Tai and Todd (15) or the thin-layer separation of carotenoids in soybean mutants by Keck *et al.* (13), allowed information on individual carotenoids and Chl to be determined. More recently, development of efficient liquid chromatographic methods of pigment separation (5, 6, 8) have been used by us and others to elucidate further the effects of genetic control on pigment development in tobacco (5), soybeans (7), and peanuts (7). In a previous study (7), we showed that a number of soybean and peanut mutants could be fitted into a progressive series of chloroplast pigment development. We speculated that these pigment-deficient mutants might represent evolutionary or arrested stages of chloroplast development. This is consistent with the suggestion of Benedict and Ketring (2) that mutation in virescent peanut leaves results in the limited synthesis of a factor necessary for cell and chloroplast differentiation. We were curious to know how this genetic control of pigment deficiency was expressed and how this expression was affected by plant and leaf maturity. To do this, we needed to be able to separate effects due to LA[1] from those due to PA. For instance, if one takes samples of leaves from the same node over a period of time, both LA and PA increase and their effects overlap. This overlap can be resolved by choosing other samples which correspond to the same node samples in LA but differ in PA. This is possible if one chooses samples from different nodes. Comparison of these samples allows us to know if pigment development is directly related to LA and if this relation holds even when the leaves are taken from different nodes at different times. The comparison between same node and different node development is especially useful when comparing geotypes which have various rates of plant development, since plant effects are readily apparent. For instance, if a 7-day-old leaf from the first trifoliolate and a 7-day-old leaf from the fifth trifoliolate have similar pigment composition in three genotypes but differ considerably in a fourth, the discrepancy is a function of genetic control. If at a more mature stage all four genotypes are similar, the genetic effect is overridden by PA. We intended, therefore, to obtain quantitative information on the composition of chloroplast pigments in a wide range of mutant genotypes (all in the genetic background of the Clark variety) throughout a growing season and to compare the development of pigments in the mutants to the normal or parent plant. The mutant:parent ratio obtained would be a quantitative measure of individual gene expression as a function of plant and leaf maturity.

EXPERIMENTAL

Genotype Description. Several genes controlling abnormal Chl development were selected for study from the USDA's Genetic Type Collection as listed below:

Strain Designation	Genotype	Plant Appearance
T139	y_3y_3	Green plant parts turn yellowish green shortly after development
T138	$y_7y_7y_8y_8$	Light yellow-green seedlings soon become green
T135	y_9y_9	Bright yellow-green color throughout growing season turning green in maturity
T219H	$Y_{11}y_{11}$	Bright yellow-green color throughout growing season turning greener in maturity

The original genetic studies on these traits were made by R. L.

[1] Abbreviations: LA, leaf age; PA, plant age; UF, unifoliolate leaf; TF, trifoliolate leaf; RLA, relative leaf age.

Table I. *Pigment Content of Parent Soybean Variety as a Function of Plant and Leaf Maturity*

Sample	PA	RLA	Pigments					
			Clark					
			Neoxanthin	Violaxanthin	Lutein	Chl *b*	Chl *a*	Carotene
	days		*nmol/cm²*					
Group I								
5th TF (1)	35	0	1.44 ± 0.19	2.39 ± 0.83	5.81 ± 0.54	11.03 ± 2.09	30.21 ± 5.03	3.52 ± 0.44
1st TF	29	7	1.92 ± 0.05	3.49 ± 0.04	6.17 ± 0.53	13.14 ± 1.27	43.97 ± 3.57	5.98 ± 0.72
UF	22	9	2.15 ± 0.28	3.44 ± 1.32	8.40 ± 1.05	15.00 ± 0.83	51.24 ± 26.6	6.91 ± 0.76
Group II								
5th TF (1)	35	0	1.44 ± 0.19	2.39 ± 0.83	5.81 ± 0.54	11.03 ± 2.09	30.21 ± 5.03	3.52 ± 0.44
5th TF (2)	43	8	1.98 ± 0.44	3.46 ± 0.84	8.71 ± 0.81	15.07 ± 0.99	47.47 ± 3.12	6.25 ± 0.22
5th TF (3)	48	13	2.78 ± 0.07	4.17 ± 0.50	9.72 ± 0.47	18.54 ± 2.16	56.64 ± 1.82	7.56 ± 0.49
5th TF (4)	55	20	2.82 ± 0.09	3.74 ± 0.13	9.45 ± 0.49	19.90 ± 0.66	59.17 ± 1.16	7.29 ± 0.30
5th TF (5)	63	28	2.92 ± 0.09	2.65 ± 0.13	8.85 ± 1.19	21.31 ± 2.31	63.93 ± 4.91	8.06 ± 0.54

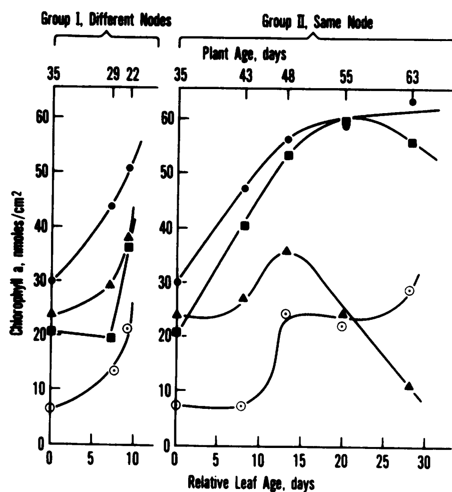

FIG. 1. Chl *a* content (nmol/cm²) as a function of LA and PA in the soybean variety Clark (C). CL1 (●), $Cy_7y_7y_8y_8$ (■), Cy_3y_3 (▲), Cy_9y_9 (⊙).

Bernard and M. G. Weiss (4). The origins of these strains are as follows:

T139—Found in the variety Illini by Dr. Brunson in Kansas about 1936.

T138—Unknown. Obtained by Dr. C. M. Woodworth, University of Illinois prior to 1935.

T135—Found in the variety Illini in 1938, presumably by Dr. Woodworth.

T219—Found in Richland X Linman 533 in 1941 at Iowa State University.

A normal green soybean variety, Clark (CL1) was chosen for study. Clark is of maturity group IV and was widely grown in the southern Midwest from 1955 to 1974. Isolines of the abnormal Chl types were developed by backcrossing to Clark as indicated below:

Strain Designation	Genotype	Backcross
L63-2346	y_3y_3	$Clark^6 \times T139$
L63-1792	$y_7y_7y_8y_8$	$Clark^6 \times T138$
L69-4755	y_9y_9	$L6^6 \times T135$
L72-1937	$Y_{11}y_{11}$	$Clark^6 \times T219$

L6 is an isoline of Clark with single genes for resistance to the diseases bacterial pustule leaf spot (*rxp*) and *Phytophthora* root rot (*Rps_1*) selected from ($Clark^8$ × CNS) × ($Clark^8$ × Blackhawk).

These isolines are homozygous for the transferred genes except for $Y_{11}y_{11}$ which is lethal; therefore, the line is maintained by bulking seeds from the distinctive $Y_{11}y_{11}$ plants. In some cases, the segregating F_2 plants from the final backcross made in producing the lines were studied; in other cases, the F_2 of crosses between selected isolines was studied.

Plants and Sample Preparation. All genotypes were planted in an outdoor plot on May 15, 1979, in Peoria, Illinois, and sampled as follows: the UF at 22 days, first TF at 29 days, fifth TF_1 at 35 days, fifth TF_2 at 43 days, fifth TF_3 at 48 days, fifth TF_4 at 55 days, and fifth TF_5 at 63 days. RLA was calculated from the day of leaf expansion (12) to sampling.

For each sample time, eight leaves from separate plants were picked, and a small disc was removed from each leaf with a paper punch (eight discs = 2.54 cm²). This procedure was repeated twice more for a total of three replicates. Each of three replicates was processed and analyzed by our method of sample preparation and chromatography[2] (8). Mean pigment values and standard deviations were calculated from the data.

RESULTS AND DISCUSSION

The samples comprise two groups. Group I is composed of three samples picked from different nodes of the plant at various plant and leaf ages. These samples are: UF (RLA, 9 days; PA, 22 days), first TF (RLA, 7 days; PA, 29 days), and fifth TF (RLA, 0 days; PA, 35 days).

The second group is composed of five samples all taken from the fifth TF at approximately weekly intervals. These samples are: fifth TF_1 (RLA, 0 days; PA, 35 days), fifth TF_2 (RLA, 8 days; PA, 43 days), fifth TF_3 (RLA, 13 days; PA, 48 days), fifth TF_4 (RLA, 20 days; PA, 55 days), and fifth TF_5 (RLA, 28 days; PA, 63 days). In group I samples, RLA does not correspond with PA, whereas in group II samples, RLA and PA both increase at the same rate.

The chloroplast pigment contents (nmol/cm²) for the normal green parent plant, Clark (CL1) as a function of plant and leaf maturity, are shown in Table I. Similar data for Chl *a* only are illustrated in Figure 1 for CL1 and three of its pigment-deficient genotypes, y_7y_7-y_8y_8, y_3y_3, and y_9y_9.

Table I and Figure 1 demonstrate same node and different node pigment development as a function of increasing LA. Group I (fifth TF_1; first TF, UF) has RLA from 0 to 9 days and illustrates the result of opposing trends in LA and PA whereas group II (fifth TF_1; fifth TF_5) has RLA from 0 to 28 days and illustrates their

[2] The mention of firm names or trade products does not imply that they are endorsed or recommended by the United States Department of Agriculture over other firms or similar products not mentioned.

Table II. *Effects of LA and PA on Chl Content of the Mutants*

Sample	RLA days	PA days	Chl *a*			
			Genotype			
			CL1	$Cy_7y_7y_8y_8$	Cy_3y_3	Cy_9y_9
	days		*nmol/cm^2*			
1st TF	7	29	44	20	29	14
5th TF	7	42	45	39	26	7
UF	9	22	51	37	38	26
5th TF	9	44	53	44	29	11

cooperation. It is evident from Table I and Figure 1 that pigment composition of the parent plant (CL1) in group I is directly related to LA and is independent of or less dependent on PA. Pigment accumulation for CL1 in group II samples is also directly related to age, but a plateau is reached when Chl *a* becomes greater than 60 nmol/cm^2. As in group I samples, the Chl *a* content is an indicator of leaf maturity in the early period of Chl *a* accumulation.

Although PA has small effect on pigment accumulation in aging leaves of normal plants, it exerts strong control in certain pigment-deficient genotypes. These effects are apparent in Figure 1. In group I samples, each of the three pigment-deficient genotypes, y_3y_3, $y_7y_7y_8y_8$, and y_9y_9 exhibits some lag in Chl accumulation with increasing RLA in comparison to the normal parent plant. One genotype ($y_7y_7y_8y_8$), however, exhibits a particularly strong depression of pigment formation in the first TF (RLA 7 days), essentially a lag in chloroplast pigment development. No such lag is evident for this genotype in group II samples where leaf aging proceeds in a more mature plant. The PA control of pigment deficiency is also visually evident in the life cycle of the $y_7y_7y_8y_8$ genotype. The cotyledons and UF leaves appear nearly normal, but the first TF emerges yellow and pigment-deficient. The recovery to normal is rapid, however, and parallels that of the normal plant thereafter.

The genotype y_9y_9, which shows a small lag in group I samples (young plant), shows a more pronounced lag period in group II samples (older plant). The extent of pigment depression is as large as that shown by $y_7y_7y_8y_8$ but occurs later. However, unlike $y_7y_7y_8y_8$, the genotype y_9y_9 experiences only moderate recovery with increasing age. The genotype y_3y_3 exhibits a small lag in group I samples and a slightly greater lag in group II samples. It is difficult, however, to separate the effect of lag from that of premature senescence, which occurs midway through greening and causes a rapid plunge in pigment levels.

The relationships among LA, PA, and genetics can be further clarified by reference to Table II, where samples of the same LA but different PA are compared for selected genotypes. Chl *a* values for 7- and 9-day RLA samples are taken directly from group I and extrapolated from the curves in Figure 1 for group II samples. The relatively small effect of PA on the parent plants is evident. The other genotypes show the early deficiency of $y_7y_7y_8y_8$ and the later deficiency of y_3y_3 and y_9y_9.

Thus, the trends of pigment development as a function of maturity shown by the various pigment-deficient genotypes used in this study follow pathways controlled by their respective genes. To demonstrate further the effects of genetic control on all chloroplast pigments, it is helpful to normalize the pigment content of each genotype against its parent type. This minimizes the effects of leaf position in the canopy, LA, and weather and growth conditions. Thus, the ratio of mutant/parent gives an indication of how each genotype is synthesizing pigments during maturation in comparison to the normal parent plants. A ratio of 1.0 indicates that there is no discernible difference between mutant and parent. Lower ratios signify lower pigment content in the mutants. Table III presents these data for increasing PA with subgroupings of maturity and genotype for individual pigments. Inasmuch as the effects of LA and PA on pigment content are cancelled by the normalizing procedure, the data in this table reflect the genetic effects. Nevertheless, genetic control of pigment deficiency may be more strongly expressed in a young leaf or young plant than in an old one, or vice versa. Examination of the data in Table III gives some partial answers to this problem. For the genotype

Table III. *Mutant/Parent Pigment Ratios as a Function of Plant and Leaf Maturity*

Sample	$Cy_7y_7y_8y_8$	Cy_3y_3	Cy_9y_9	$CY_{11}y_{11}$	RLA	PA
		ratio			*days*	
Neoxanthin						
UF	0.80	0.78	0.40	0.44	9	22
1st TF	0.43	0.73	0.25	0.32	7	29
5th TF (1)	0.77	0.76	0.19	0.34	0	35
5th TF (2)	0.97	0.66	0.14	0.24	8	43
5th TF (3)	0.87	0.68	0.25	0.22	13	48
5th TF (4)	0.99	0.39	0.28	0.29	20	55
5th TF (5)	0.97	0.16	0.41	0.26	28	63
Violaxanthin						
UF	0.60	0.77	0.55	0.73	9	22
1st TF	0.53	0.93	0.58	0.60	7	29
5th TF (1)	0.92	0.94	0.51	0.89	0	35
5th TF (2)	1.03	0.78	0.20	0.41	8	43
5th TF (3)	0.94	0.71	0.35	0.43	13	48
5th TF (4)	1.00	0.59	0.54	0.62	20	55
5th TF (5)	0.96	0.28	0.46	0.38	28	63
Lutein						
UF	0.88	1.05	0.92	0.89	9	22
1st TF	0.45	0.79	0.54	0.58	7	29
5th TF (1)	0.86	0.86	0.60	0.66	0	35
5th TF (2)	0.86	0.72	0.58	0.70	8	43
5th TF (3)	0.92	0.73	0.52	0.51	13	48
5th TF (4)	0.96	0.52	0.43	0.55	20	55
5th TF (5)	1.05	0.50	0.52	0.42	28	63
Chl *b*						
UF	0.73	0.69	0.25	0.24	9	22
1st TF	0.46	0.63	0.20	0.21	7	29
5th TF (1)	0.76	0.78	0.13	0.26	0	35
5th TF (2)	0.87	0.50	0.08	0.14	8	43
5th TF (3)	1.00	0.61	0.28	0.15	13	48
5th TF (4)	1.00	0.34	0.25	0.23	20	55
5th TF (5)	0.94	0.12	0.42	0.26	28	63
Chl *a*						
UF	0.71	0.75	0.42	0.47	9	22
1st TF	0.46	0.67	0.32	0.37	7	29
5th TF (1)	0.70	0.79	0.25	0.45	0	35
5th TF (2)	0.82	0.68	0.30	0.45	8	43
5th TF (3)	0.91	0.74	0.46	0.51	13	48
5th TF (4)	0.99	0.58	0.48	0.66	20	55
5th TF (5)	0.84	0.38	0.53	0.47	28	63
Carotene						
UF	0.67	0.84	0.63	0.74	9	22
1st TF	0.43	0.74	0.47	0.56	7	29
5th TF (1)	0.64	0.84	0.52	0.72	0	35
5th TF (2)	0.82	0.68	0.30	0.45	8	43
5th TF (3)	0.91	0.74	0.46	0.51	13	48
5th TF (4)	0.99	0.58	0.48	0.66	20	55
5th TF (5)	0.84	0.38	0.53	0.47	28	63

$y_7y_7y_8y_8$, the mature UF (RLA 9 days) and the immature fifth TF_1 (RLA 0 days) both contain approximately 70 to 80% of the pigments of the normal parent (CL1). The strong PA effect mentioned earlier occurs in the first TF (RLA 7 days), which has only about 45% of the pigments of the parent. The fifth TF_2-fifth TF_5 show the rapid recovery to normal behavior. The genotype y_3y_3 generates about 70 to 80% of normal pigment levels with a slight dip in first TF and a larger one in fifth TF_2. In the fifth TF_4 and fifth TF_5 the effect of senescence becomes apparent.

It is difficult to ascertain the exact effects occurring in y_9y_9. It appears from the first three samples that pigment deficiency is expressed more strongly in young leaves than in mature ones. That is, pigment content decreases with decreasing LA (RLA). However, in the next sample, RLA starts to increase again but the pigment deficiency continues. Thus, the lag in pigment development is not only PA dependent but also becomes stronger with increasing PA. The heterozygote $Y_{11}y_{11}$ exhibits similar behavior.

A lag in Chl accumulation is also exhibited in the greening of etiolated virescent peanut seedlings exposed to continuous illumination. Benedict and Ketring (2) have shown that compared to wild-type leaves, a lag period of 72 h occurs in virescent peanut leaves before rapid Chl accumulation begins. The genetic message for lag is thus built into the original greening process and echoed in whole-plant development as well. Although light pretreatment of etiolated virescent leaves followed by a dark period allows Chl accumulation to commence rapidly upon reillumination, the dark period necessary is equal to the lag time (3). Some component synthesized during the dark period is necessary for plastic development to begin. Evidence gathered by previous investigators (14, 16) suggests that in higher plants this regulator may be phytochrome, although Holowinsky and Schiff (11) have shown that protochlorophyllide functions in this capacity in simpler systems such as *Euglena* and may be a secondary or back-up system in higher plants. Similar components may also be responsible for whole-plant development as well. The exact lag period for whole-plant pigment development is difficult to pinpoint but ranges from 20 to 30 days for $y_7y_7y_8y_8$ to 40–50 days for y_9y_9 and $Y_{11}y_{11}$.

Throughout this paper, the various genotypes have been characterized as pigment-deficient, not just Chl-deficient. All pigments are affected by the deficiency, but some more than others. The primary deficiency both in lag effects and in senescence occurs in Chl *a* and *b* and in neoxanthin. Violaxanthin, lutein, and carotene also show these effects but to a more limited extent. Since pigment deficiency in many of these genotypes can be attributed to a reduction in the light-harvesting Chl *a*/*b* protein (1), these data suggest that neoxanthin may be associated with or be a part of this pigment protein complex.

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