Proceedings of the Iowa Academy of Science

Volume 76 | Annual Issue Article 13

1969

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Abstract. Xantha form mutants of *Glycine Max* (L.) *Merril* were examined for their chlorophyll content during early seedling development. Trifoliate leaves of 8-24 day old dark green, light green and yellow plants differed significantly in chlorophyll content. Total chlorophyll content in all three phenotypes increased up to approximately the 16th day followed by a slight drop from the 16th to the 24th day. The relative proportion of chlorophyll a to chlorophyll b differed among the three leaf types.

Chloroplast pigment content of mutants of higher plants has been investigated in numerous species. Weber and Weiss (1959) described a chlorophyll mutant of soybean, *Glycine Max* (L.) *Merril,* assigned the gentic symbol y_{11}. F^2 progenies of heterozygotic plants ($Y_{11}y_{11}$) were found to segregate in a ratio of 1 dark green; 2 light green; 1 yellow. Wolf (1963) reported three types of 10 day old plants, yellow, light green and dark green, differed in total chlorophyll content and in their chlorophyll a / chlorophyll b ratio. Quantitative analysis showed the total chlorophyll content of dark green plants was approximately twice that of light green plants. Noteworthy is the appreciable amount of total chlorophyll found by Wolf in the yellow ($y_{11}y_{11}$) mutant.

The use of mutant soybean plants differing in quantities of total chlorophylls might prove rewarding in attempting to correlate photosynthetic activity with pigment concentration. To establish this relationship, it is necessary to know if there is any variation in the total chlorophyll content and chlorophyll a / chlorophyll b ratio during early seedling development. Therefore, this paper is concerned with establishing the amount of chlorophyll associated with early seedling development of dark green, light green and yellow soybean plants.

MATERIALS AND METHODS

Seeds of the F_2 progeny were obtained from General Biological Supply House Inc., Chicago, Illinois. Trifoliate leaves of the three genotypes were removed every fourth day from the 8th through the 24th day. Fresh weights of the blades were determined with an analytical balance. Each blade was cut into small pieces, and 0.5 gms was ground in a mortar and pestal with 10 ml of 85% acetone.

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The surry was suction filtered and rinsed with 40 ml of 85% acetone. The final filtrate volume was adjusted to 50 ml with 85% acetone.

Chlorophylls a and b were estimated in acetone extracts by absorbancy determinations at 663 mμ and 644 mμ using a Bausch and Lomb spectronic 20 spectrophotometer (Wolf, 1963; Kurtz and Mellor, 1966). Concentrations of chlorophyll a and b in mg per gm of tissue were calculated using the procedure of Kurtz and Mellor (1966).

chlorophyll a/gm tissue = 1.07 (A663) — 0.094 (A644)
chlorophyll b/gm tissue = 1.77 (A644) — 0.280 (A663)

The constants used in these calculations were determined by Arnon (1959) from the early work of MacKinney (1941).

Results

The F^2 progeny segregated in the expected 1 dark green; 2 light green; 1 yellow phenotypic ratio as determined from observations of 8 day cotyledons.

Analysis of chlorophyll content is given in Table 1. It is apparent that trifoliate leaves of the three types of plants differ significantly in chlorophyll content. Dark green leaves have approximately twice the total chlorophyll content as light green leaves from day 8 through day 24. Also on day 8 dark green leaves have approximately four times as much total chlorophyll content as yellow leaves. However this ratio is not maintained beyond day 8. Light green leaves have about twice the chlorophyll content on day 8 as the yellow leaves. However, this ratio is not retained on days 12-24. The total chlorophyll content in all three leave types increased up to about day 16 and then dropped slightly. However, the 12-24 day yellow leaves did not show a marked increase, containing only approximately 7-10 percent chlorophyll as compared to the dark green leaves.

The relative proportion of chlorophyll a to chlorophyll b (a/b) can be seen to differ among the three types of leaves. The a/b ratio in dark green leaves increased sharply between 8-12 days, then fell between 12-16 days and remained the same till day 24. However, in both light green and yellow leaves the a/b ratio remained relatively high from day 12 to day 24.

Discussion

Analysis of chlorophyll pigments in leaves of dark green, light green and yellow soybean plants has shown a difference between each leaf type in quantities of total chlorophylls and in chlorophyll a / chlorophyll b ratio during early seedling development. Using just the trifoliate leaves we were able to confirm the studies of

Table 1. Chlorophyll Content of Seedling Leaves[a]

Dark Green

Day	Chl a	chl b	Total chl	chl a / chl b
8	0.36	0.28	0.64	1.28
12	1.02	0.67	1.69	1.50
16	1.41	1.24	2.65	1.14
20	1.31	1.15	2.46	1.14
24	1.31	1.15	2.46	1.14

Light Green

Day	Chl a	chl b	Total chl	chl a / chl b
8	0.21	0.17	0.38	1.20
12	0.61	0.39	1.00	1.56
16	0.96	0.64	1.60	1.50
20	0.81	0.56	1.37	1.43
24	0.81	0.50	1.31	1.60

Yellow

Day	Chl a	chl b	Total chl	chl a / chl b
8	0.08	0.07	0.15	1.17
12	0.08	0.04	0.12	2.10
16	0.15	0.09	0.24	1.60
20	0.14	0.10	0.24	1.40
24	0.11	0.07	0.18	1.60

[a]Concentrations expressed as mg. chlorophyll per gm. fresh wt.

Wolf (1963) in which he related the chlorophyll content of 8 day old $Y_{,,}Y_{,,}$ (dark green genotype to the $Y_{,,}y_{,,}$ (light green) genotype. We have shown that on day 8 the chlorophyll content of dark green ($Y_{,,}Y_{,,}$) leaves to be approximately twice that in the light green ($Y_{,,}y_{,,}$) leaves. We have shown that this ratio remains through the first 24 days. The established ratio of total chlorophyll content in dark green ($Y_{,,}Y_{,,}$) leaves to yellow ($y_{,,}y_{,,}$) leaves as reported by Wolf (1963) for day 8 also remains through day 24.

While the determination of chlorophyll content in soybean mutants during early seedling development was the major objective of this investigation, it came to our attention that the general internal structure of the leaves and stems of each plant type might be modified (Wallce and Habermann, 1959). Therefore, fresh frozen cross-sections of 16 day old seedling stems 15 mm above the cotyledonary node were obtained using a I.E.C. refrigerated microtome. These sections were stained with phloroglucin – HCL. Sections of yellow ($y_{,,}y_{,,}$) stems showed reduced lignification. There was also some tendency toward reduction of tissue compactness in both yellow and light green mutants. Since the chlorophyll pigments are apparently under genetic control in soybean plants, it is reasonable

that formation of stem tissue might be dependent upon photosynthetic activity. However, Wolf (1963) has shown that the relationship of total carotenoids and total xanthophyllis in the three types of soybeans approximates that found for chlorophylls. This would appear to imply multiple function for the genes involved. Perhaps some metabolic activity other than photosynthesis is affecting stem tissue development in the yellow and light green soybean mutants.

ACKNOWLEDGEMENT

The authors wish to express their appreciation to Donald Blaker and John Bjorkquist for their work during the investigations.

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