FATTY ACIDS OF BUSH BEAN LEAF CHLOROPLASTS AND PROPLASTIDS

D. W. Newman

Department of Botany, Miami University, Oxford, Ohio

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Several investigators have analyzed higher-plant plastids for lipid materials (Comar, 1942; Straus, 1954; Zill and Harmon, 1959). Galactolipids predominate in chloroplasts, whereas phosphatidyl glycerol is the predominant phosphatide in the chloroplasts that Benson analyzed (1961). Wolf (1962) analyzed the fatty acid content of spinach chloroplasts and found linolenic acid in the highest concentration and at least eleven other fatty acids in lesser amounts. Most of the above-mentioned reports and others are concerned either with lipid changes during short-term periods of photosynthesis or with carrot-chromoplast lipids. Few investigations have dealt with the lipids as plastids break down or as plastids are converted from one type to another - comparatively long-term changes of the plastids. We have attempted to investigate the quality and quantity of some of these lipids of changing plastids.

Methods and Results

Whole chloroplasts or proplastids were isolated by sedimentation between 200 and 500 or 1,000 x g in cold 0.35 M NaCl following release of the plastids in a Waring blendor. A total lipid extract from the ethanol-boiled plastid fraction was made by the method of Folch (1957) using chloroform and methanol. Free fatty acids were isolated from the mixture by addition of a one per cent sodium carbonate solution and the aqueous and non-aqueous phases were separated (Lee and Mattick, 1961). The free fatty acids were esterified with methanolic BF_3 (Metcalfe and Schmitz, 1961). The non-aqueous phase was refluxed for two hours in ethanolic KOH under nitrogen, acidified, the fatty

acids extracted and esterified. Analyses of the esterified fatty acids were made using two different gas chromatograph column packings (Apiezon L on Celite and ethylene glycol-succinate polyester on Celite). The fatty acids were collected, brominated and rechromatographed in order to identify unsaturated materials. Highly purified standard mixtures of known quality and quantity were used to determine retention times of the columns and correction factors and linearity of response of the detectors. Chlorophyll analyses were made on freshly prepared plastids by the method of Koski (1950).

Bean plants were grown in the light or in the dark; in vermiculite irrigated with a complete mineral nutrient, with a minus-iron mineral nutrient or with distilled water. Two different experiments were run using plants irrigated with a complete mineral nutrient or a minus-iron mineral nutrient.

Table 1 summarizes the ratios of saturates to unsaturates and of C16 to C18 fatty acids and the chlorophyll contents. Plastids with a reduced chlorophyll content, in each experiment, exhibited a consistent increase in the ratio of saturates to unsaturates and an increase in the ratio of C16 to C18 fatty acids. No attempt should be made to correlate data from one experiment to another, since the quantity of fatty acids varies with respect to the maturity of the leaf from which the plastids are isolated. In another experiment the plastids isolated from first, second, third and fourth node leaves subjected to a complete or minus-iron nutrient were analyzed. Table 2 gives the ratios of saturates to unsaturates, of C16 to C18 fatty acids, and of stearic to C18 unsaturates along with the chlorophyll contents of the isolations. The minus-iron treated plants exhibited a consistently higher ratio of plastid saturates to unsaturates, a higher ratio of stearic acid to C18 unsaturates and a higher ratio of C16 to C18 fatty acids, except for plastids isolated from first node leaves. For both types of treatments, a definite relationship was exhibited between the relative quantity of fatty acid and the maturity of the leaf from which the plastids were isolated (Table 2, Figure 1). Fatty acids with eighteen carbons seemed to decrease, comparatively, with maturity, while C16 and C14 fatty acids increased slightly. However, these rates of

change with respect to maturity were more marked for plastids isolated from complete nutrient-irrigated plants than from minus-iron nutrient-irrigated plants.

TABLE 1

Ratios of saturates to unsaturates and C16 to C18 fatty acids and chlorophyll contents of plastids.

Sample	Saturates/ unsaturates	C16/C18	Chlorophyll content (mg per isolation)
Light-grown	0.80	0.78	20.1
Dark-grown	1.09	0.99	0.04
Nutrient-grown	0.39	0.27	35.2
Distilled water-grown	0.70	0.47	13.6
Nutrient-grown	0.63	0.59	...
-Fe - grown	0.70	1.12	...

TABLE 2

General fatty acid composition of plastids isolated from complete or minus-iron irrigated plants.

Sample	Node (bottom to apex)	Total C14, C16, C18 acids (mg x 2 x 10^{-2}) per isolation	saturates/ unsaturates	C16/ C18	Stearic/ C18 unsaturates	Chlorophyll (mg/isolation)
Complete	1	227	0.75	0.82	0.21	19.0
	2	98.7	0.53	0.37	0.19	16.8
	3 & 4	662	0.36	0.29	0.13	33.3
-Fe	1	145	0.91	0.67	0.27	3.4
	2	120	0.73	0.62	0.22	10.2
	3 & 4	218	0.65	0.48	0.20	14.8

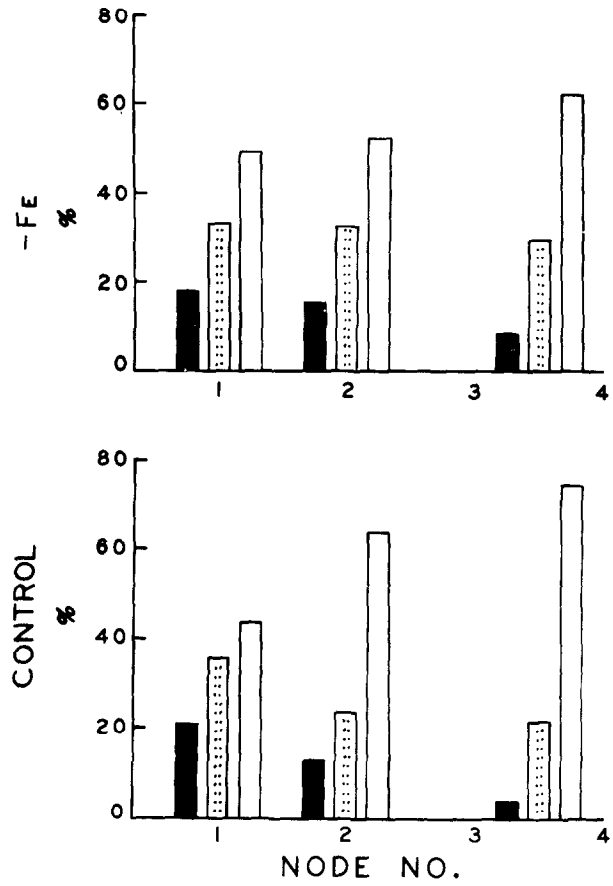

Fig. 1. Percentages of C14 (solid bars), C16 (dotted bars) and C18 (open bars) fatty acids of plastids isolated from plants grown in complete and minus-iron mineral nutrients.

Discussion

It is apparent from these data that the quality of fatty acids of bush bean leaf plastids is greatly dependent upon the state of maturity of the tissue, as well as the environment to which the plastids were subjected, prior to isolation. In all cases, more mature tissue contained plastids with comparatively less unsaturated fatty acids. Mead (1960) concluded that hydrogenation of long-chain unsaturated fatty acids probably occurs in plant tissues. However, the above data do not indicate whether the increase of saturates in more mature tissues was due to hydrogenation of existing fatty acids or a reconstruction from lower-chain moieties.

References

Benson, A. A. 1961. In: Life and Light, Johns Hopkins Press, Baltimore, p. 392.
Comar, C. L. 1942. Botan. Gaz. 104, 122.
Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. J. Biol. Chem. 226, 497.
Koski, V. M., C. S. French, and J. H. C. Smith. 1950. Arch. Biochem. Biophys. 29, 339.
Lee, F. A., and L. R. Mattick. 1961. J. Food Sci. 26, 273.
Mead, J. F. 1960. In: Lipide Metabolism, John Wiley and Sons, N.Y., p. 52.
Metcalfe, L. D., and A. A. Schmitz. 1961. Anal. Chem. 33, 363.
Straus, W. 1954. Exptl. Cell Research 6, 392.
Wolf, F. T., J. G. Coniglio, and J. T. Davis. 1962. Plant Physiol. 37, 83.
Zill, L. P., and E. A. Harmon. 1959. Fed. Proc. 18, 359.