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THE EFFECT OF MINERAL DEFICIENCY AND LEAF AGE ON THE NITROGEN AND CHLOROPHYLL CONTENT OF SPINACH CHLOROPLASTS

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SUMMARY

The nitrogen and chlorophyll content of chloroplasts from spinach plants varies with leaf age and the nutritional status of the plant. Both chlorophyll and nitrogen content of the chloroplasts increase with leaf age when plants are grown on full nutrient. Chloroplasts from manganese- and iron-deficient plants contain less chlorophyll but similar amounts of nitrogen to chloroplasts from plants grown on full nutrient. Chloroplasts from sulphur- and potassium-deficient plants contain lower amounts of both chlorophyll and nitrogen than control plants.

INTRODUCTION

There are few reports in the literature of the nitrogen and chlorophyll content of chloroplasts. This is mainly due to the difficulty of isolating intact chloroplasts which have retained both their chlorophyll and water-soluble protein.

MENKE[1] isolated Antirrhinum chloroplasts in non-aqueous media and measured $11.0 \cdot 10^{-12}$–$12.2 \cdot 10^{-12}$ g protein per chloroplast. The total dry weight of the chloroplast was $24.6 \cdot 10^{-12}$ g which was considerably more than the dry weight of bean chloroplasts which have been found to be $7.9 \cdot 10^{-12}$ g (ref. 2). The bean plastids were isolated in aqueous media to which formaldehyde had been added to stabilize the chloroplast structure. These workers recorded a protein content of $5.3 \cdot 10^{-12}$ g for the plastids of light-grown plants. These isolation techniques were also used by MARGULIES[3] in his work on bean plastids in which he recorded protein nitrogen levels of $7.8 \cdot 10^{-12}$ g for light-grown plants.

Conditions have been characterized for the isolation of spinach chloroplasts in aqueous media which retain both the chlorophyll and water-soluble protein[4]. Such methods have the advantage over non-aqueous isolation procedures in that the chloroplasts retain their chlorophyll and are easily recognized when preparations are examined under the microscope.

The present paper provides information on the chlorophyll and nitrogen content of chloroplasts isolated from leaves of spinach plants grown on full nutrient or in solutions lacking either iron, manganese, sulphur or potassium. Plants deficient in these elements were examined because it is well known that iron, manganese and sulphur deficiencies cause chlorosis in spinach plants, while potassium deficiency has recently been shown to significantly increase the chlorophyll content of the leaves[5].

METHODS

Plants were grown in nutrient solution under controlled conditions[5]. When plants showed deficiency symptoms chloroplasts were isolated and the nitrogen and chlorophyll content measured by previously described methods[4].

Chloroplast counting

Chloroplasts were counted in a Petroff–Hausser bacteria counter 0.0025 mm^2 by 0.02 mm deep (C.A. Hausser and Son, Philadelphia) after dilution with suspension medium containing 3% glutaraldehyde or 2 mg per ml of bovine serum albumin. Dilution was adjusted to give about 200 chloroplasts over the calibrated area of the bacteria counter. 0.1% of a neutral detergent (Divo, a product of Diversey A/Asia Pty., Lane Cove, N.S.W.) was added to chloroplasts treated with glutaraldehyde to aid dispersion.

RESULTS

The nitrogen and chlorophyll content of chloroplasts from plants grown on full nutrient and on solutions deficient in manganese are shown in Table I. Chloroplasts isolated from plants showing deficiency symptoms contained half the chlorophyll of chloroplasts from plants grown on full nutrient but contained the same amount of nitrogen. This resulted in a nitrogen to chlorophyll ratio for chloroplasts from manganese-deficient plants twice that of chloroplasts from plants grown on full nutrient. In a large number of experiments the nitrogen and chlorophyll ratios of full nutrient and manganese-deficient plants were measured. The mean values were 4.07 ± 0.15 (mean of 30 replicates) for chloroplasts from plants showing manganese-deficiency symptoms and 1.94 ± 0.07 (mean of 53 replicates) for chloroplasts from plants grown on full nutrient.

In plants showing manganese-deficiency symptoms leaf position had little effect on the nitrogen and chlorophyll content of the chloroplasts and the nitrogen to chlorophyll ratio was constant (Table II). However, leaf position had a marked effect

TABLE I

EFFECT OF MANGANESE DEFICIENCY ON THE CHLOROPHYLL AND NITROGEN CONTENT OF CHLOROPLASTS

Chlorophyll per chloroplast values are the means of 7 experiments and nitrogen per chloroplast values are means of 5 experiments. Plants of different age and different leaves were used in each experiment.

Treatment	*Chlorophyll per chloroplast (pg)*	*Nitrogen per chloroplast (pg)*
Full nutrient	0.61 ± 0.21	1.15 ± 0.32
Manganese deficient	0.29 ± 0.04	1.10 ± 0.15

TABLE II

EFFECT OF LEAF POSITION ON NITROGEN AND CHLOROPHYLL CONTENT OF MANGANESE-DEFICIENT CHLOROPLASTS

Leaves 1 and 2 were the basal pair, 3 and 4 the intermediate pair, 5 and 6 the apical pair of leaves. Each value is the mean of 4 replicates.

Leaf position	*Total nitrogen per chloroplast (pg)*	*Chlorophyll per chloroplast (pg)*	*Nitrogen to chlorophyll ratio*
Leaves 1 and 2	1.083	0.281	3.86
Leaves 3 and 4	1.017	0.244	4.16
Leaves 5 and 6	0.964	0.225	4.28

TABLE III

EFFECT OF IRON DEFICIENCY ON NITROGEN AND CHLOROPHYLL CONTENT OF CHLOROPLASTS

Leaves 1 and 2 were the basal pair, 3 and 4 the intermediate pair and 5 and 6 the apical pair of leaves. Each value is the mean of 4 replicates.

Nutritional treatment	*Leaf No.*	*Total nitrogen per chloroplast (pg)*	*Chlorophyll per chloroplast (pg)*	*Nitrogen to chlorophyll ratio*	*Chlorophyll A to B ratio*
Minus iron	1 and 2, green	0.65	0.161	2.63	4.17
	3 and 4, mottled	0.51	0.093	4.61	4.83
	5 and 6, yellow	0.90	0.073	12.41	6.35
Control	1 and 2	0.71	0.348	2.05	4.01
	3 and 4	0.54	0.299	1.80	4.01
	5 and 6	0.45	0.217	2.06	4.20

on both the nitrogen and chlorophyll content of chloroplasts isolated from plants grown on solutions deficient in iron and on full nutrient (Table III). Iron-deficient plants showed a marked gradation in the deficiency symptoms with leaf position as the basal leaves remained green, the intermediate leaves became mottled and the top pair of leaves were yellow. Values obtained for the nitrogen content of the chloroplasts isolated from opposite leaf pairs of iron-deficient plants showed no clear trend whereas the chlorophyll content decreased and in consequence the nitrogen to chlorophyll ratio increased. Likewise the chlorophyll A to B ratio increased. The nitrogen and chlorophyll content of chloroplasts from paired leaves from plants grown on full nutrient increased with increasing leaf age but the nitrogen to chlorophyll ratio and the chlorophyll A to B ratio remained constant.

Sulphur deficiency decreased the nitrogen and chlorophyll content of the chloroplasts but had little effect on the nitrogen to chlorophyll ratio or the chlorophyll A to B ratio (Table IV). Similarly potassium deficiency decreased the nitrogen and chlorophyll content of chloroplasts and had no effect on the nitrogen to chlorophyll ratio or the chlorophyll A to B ratio (Table V).

TABLE IV

EFFECT OF SULPHUR DEFICIENCY ON THE NITROGEN AND CHLOROPHYLL CONTENT OF CHLOROPLASTS

Each value is the mean of 4 replicates.

Nutritional treatment	*Plant fresh wt. (g)*	*Total nitrogen per chloroplast (pg)*	*Chlorophyll per chloroplast (pg)*	*Nitrogen to chlorophyll ratio*	*Chlorophyll A to B ratio*
Minus sulphur	0.96	0.227	0.0998	2.25	4.85
	1.16	0.209	0.103	2.04	3.86
	2.50	0.207	0.158	1.31	3.59
Control	11.50	0.601	0.380	1.58	3.52
	12.50	0.726	0.420	1.73	3.84

TABLE V

EFFECT OF POTASSIUM DEFICIENCY ON THE NITROGEN AND CHLOROPHYLL CONTENT OF CHLOROPLASTS

Each value is the mean of 4 replicates.

Treatment	*Plant fresh wt. (g)*	*Total nitrogen per chloroplast (pg)*	*Total chlorophyll per chloroplast (pg)*	*Total nitrogen to total chlorophyll ratio*	*Chlorophyll A to B ratio*
Minus potassium	0.57	0.441	0.261	1.68	3.55
	0.16	0.505	0.200	2.52	3.36
Control	7.20	0.794	0.413	1.93	3.84
	5.10	0.928	0.453	2.02	3.97

DISCUSSION

The striking difference between control and manganese-deficient plants is the amount of chlorophyll per chloroplast, manganese-deficient plants having only half as much as control plants (Table I). This difference is due to a lower chlorophyll concentration in chloroplasts from manganese-deficient plants since both have the same amount of nitrogen and are of similar size. In this connection it is of interest that manganese-deficient plants have a greatly reduced lamella system[6]. Assuming a protein nitrogen content of 16% (ref. 7) the protein content of spinach chloroplasts from control and manganese-deficient plants was about 6.25 pg. The greater variability observed with control chloroplasts as compared with chloroplasts from manganese-deficient plants was almost certainly due to the fact that chloroplasts from leaves of control plants differed with age of leaf (Table III), whereas leaf age effects tend to be suppressed in manganese-deficient plants (Table II).

Chloroplasts from potassium-deficient plants were examined since VESK and co-workers[8] had observed no decrease in chloroplast size in deficient plants while more recently it has been shown that the chlorophyll content of leaves of spinach plants deficient in this element is increased[5]. Chloroplasts isolated from potassium-deficient plants were found to be smaller and their nitrogen and chlorophyll content was less

(Table V), than chloroplasts from plants grown in full nutrient. The dark green colour of potassium-deficient tissue therefore is not due to an increase in the chlorophyll content of the chloroplasts but to a higher density of chloroplasts in the tissue and is almost certainly associated with potassium-deficient spinach plants having leaf cells of reduced size.

This series of experiments indicates that the nitrogen and chlorophyll content of chloroplasts is not constant for a plant species. Leaf age and nutritional status have been shown to affect the values obtained for spinach. The nitrogen and chlorophyll content of chloroplasts from plants grown on full nutrient generally increased with increasing leaf age. Chloroplasts from plants deficient in manganese, iron, sulphur and potassium had less chlorophyll than plants grown in full nutrient. While the chloroplasts of sulphur- and potassium-deficient plants had less nitrogen than those of control plants. This latter effect was similar to that reported by MARGULIES[3,9] who found that treatment with chloramphenicol and lack of light gave rise to plastids with a reduced nitrogen content.

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