Effect of Manganese Deficiency on Chloroplasts of Lemon Leaves

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Abstract
Manganese deficiency in chloroplasts of Eureka lemon leaves resulted in 23% and 40% increase of chloroplast nitrogen and protein, respectively, on a chlorophyll unit basis. Acrylamide gel electrophoresis carried out on extracts of these chloroplasts disclosed also qualitative differences between the normal and deficient leaves. Calculated on chloroplast N basis there is an increase of 17% in the chloroplast protein under Mn deficiency. This increase apparently indicates a more intense protein synthesis in the Mn deficient chloroplasts. Hill activity of the —Mn leaves was about one-third of the analog control leaves. Manganese infiltration into detached but intact leaves restored the activity in the —Mn leaves up to 70% of the control. Lemon leaves affected by other macro- and micro-nutrient deficiencies did not respond to the manganese infiltration; therefore, the use of this infiltration method is suggested for the evaluation of the manganese nutrition status of citrus and probably other higher plants.

Introduction
In a previous paper (Lerer and Bar-Akiva 1976) we reported on the increase of nitrogen constituents, and among them the protein fraction, in manganese-deficient leaves. These findings referred to the cytoplasmic parts of the leaves. In this paper we report on measurements of chloroplast nitrogen and protein.

It is well documented that the oxygen evolving system (Hill reaction) of the chloroplasts is a manganese-containing entity (Anderson et al. 1964, Cheniae 1970). It is assumed that the active manganese is associated with protein (Radmer and Kok 1975) in this entity, which has been isolated and partially purified (Cheniae and Martin 1967). The possibility to use the Hill reaction as an indicator for assessing manganese requirements of higher plants was proposed by Bar-Akiva (1971) and by Basiouny and Biggs (1976), but not previously explored. In the present paper we also present the first results of using Hill reaction activity for this purpose with lemon plants.

Abbreviation: Hepes, N-2-Hydroxyethylpiperazine-N-2-ethansulphonic acid.

Materials and Methods

Plant material. Eureka lemon seedlings (Citrus limon (L.) Burm. f.), 2 to 3 months old, were transferred to aerated water culture and grown in a greenhouse (natural day light). The composition and purification of the nutrients used for the culture followed the procedure described by Hewitt (1966).

Preparation of chloroplasts. In a Virtis homogenizer, working at top speed four times for 3 s, 1 g fresh washed leaves were macerated in 20 ml of Hepes grinding medium (Cockburn et al. 1968). The suspension was filtered through nylon cloth and centrifuged at 200 g for 5 min. The supernatant was collected and recentrifuged at 1500 g for 30 min. The precipitate was dissolved in 5 ml Hepes suspension medium (Cockburn et al. 1968).

Assay of Hill reaction. By a method which is basically a modification of the procedure described by Spencer and Possingham (1960), 0.2 ml of chloroplast suspension and 4 ml of H₂O were mixed with 2 ml of 2,6-dichlorophenolindophenol dye solution (0.1 mM 2,6-dichlorophenolindophenol 0.01 M KCl, 0.04 M potassium phosphate buffer, pH 6.5). Changes in absorbance (ΔA) were recorded at 620 nm before and after illumination (500 W tungsten lamp) of the reaction mixture for 5 min.

Chlorophyll determination was by acetone extraction according to Arnon (1949).

Chloroplast protein. Total chloroplast protein was estimated by the Lowry assay (Lowry et al. 1951) in the precipitate of the chlorophyll acetone extraction.

Total nitrogen determination. This determination was carried out by Kjeldahl digestion of dried leaves, and with an autoanalyzer (Technicon Control Inc.), according to Clare and Stevenson (1964).

Chloroplast nitrogen was determined by Kjeldahl digestion to an evaporated aliquot of the chloroplast suspension, and with an autoanalyzer as in total nitrogen determination. Acrylamide gel electrophoresis of chloroplast soluble proteins. The 1500 g precipitate of chloroplasts was
suspended with 2 ml Hepes suspension medium; 8 ml freeze-
cold acetone was added and left overnight at 4 ± 1°C. After
centrifugation at 3100 g, the precipitate was dissolved with 2
ml HCl-Tris buffer 0.05 M, pH 7.5, and centrifuged at
18,000 g. An aliquot of the supernatant was taken for acryl-
amide gel electrophoresis according to Davis (1964). Staining for peroxidase isoenzymes was carried out according
to Sagiv and Bar-Akiva (1972).

Manganese infiltration. Infiltration was carried out in
excised intact leaves under vacuum suction, as described by
Achituv and Bar-Akiva (1976).

Results

Total nitrogen and protein of the chloroplast

Total chloroplast protein and nitrogen content increased
by about 40% and 25%, respectively, in the —Mn chloro-
plasts as compared with the control, expressed on a
chlorophyll unit basis (Table I). The percentage of the
chloroplast nitrogen out of the total leaf nitrogen decreased
by almost 30% (Table 1). Although calculation on a leaf
weight basis is not very common when dealing with chloro-
plasts, because of the possibility of breakdown in the
deficient leaf and different yield efficiency, it can be seen that
on a weight basis, the chloroplast protein level did not change, while the nitrogen content decreased in the —Mn
chloroplast compared with the control (Table 1).

The soluble protein fraction was separated by means of
acrylamide gel electrophoresis. Generally, the gels showed
more intense color in the extract from the —Mn chloro-
plasts than in the control (Figure 1). The increase was not
the same with all the bands, but none of them decreased
under manganese deficiency conditions. The chloroplast
peroxidase isoenzymes showed a similar pattern (Figure 1).

Chlorophyll and Hill reaction studies

The overall chlorophyll content in the —Mn chloroplasts
was 62% of the control (Table 2). The chlorophyll a/b ratio

Table 1. Chloroplast nitrogen and protein in differentially treated lemon leaves. Mean of 4–5
replications. * Significant at the 5% level. Chl, chlorophyll.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chloroplast protein</th>
<th>Chloroplast nitrogen</th>
<th>Chloroplast N % of total N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/g fr. wt.</td>
<td>mg/mg Chl</td>
<td>mg/g fr. wt.</td>
</tr>
<tr>
<td>Control</td>
<td>5.5</td>
<td>10.1</td>
<td>0.91</td>
</tr>
<tr>
<td>—Mn</td>
<td>5.6</td>
<td>14.1</td>
<td>0.78</td>
</tr>
<tr>
<td>SE</td>
<td>0</td>
<td>0.87</td>
<td>-</td>
</tr>
<tr>
<td>Level of significance</td>
<td>N.S.</td>
<td>*</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Table 2. Hill activity and chlorophylls of the —Mn and the control chloroplasts. (Means of 10–12
replications. ** Significant at the 1% level. Absolute values (mg Chl/g fr. wt.) for Chl a = 0.71; for
Chl b = 0.61.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hill activity at 620 nm</th>
<th>Chl a/b</th>
<th>Chl a %</th>
<th>Chl b %</th>
<th>Total Chl %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.8</td>
<td>1.11</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>—Mn</td>
<td>4.8</td>
<td>0.98</td>
<td>60.7</td>
<td>68.3</td>
<td>62.2</td>
</tr>
<tr>
<td>SE</td>
<td>1.5</td>
<td>0.04</td>
<td>12.6</td>
<td>9.1</td>
<td>14.5</td>
</tr>
<tr>
<td>Level of significance</td>
<td>**</td>
<td>**</td>
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</table>
decreased by about 13%. Calculating the percentage of the chlorophylls $a$ and $b$ in the $-\text{Mn}$ chloroplasts from their level in the control chloroplasts, indicates a more intense decrease of the chlorophyll $a$ (Table 2).

The Hill activity in the $-\text{Mn}$ chloroplasts was less than one-third of the control (Table 2). The specificity of the manganese effect on the Hill reaction was tested by measuring the rate of Hill activity restoration after manganese infiltration into the detached but intact leaves (referred to as ‘induced’ activity; Figure 2A). In order to eliminate the infiltration effect, distilled water was infiltrated into another part of the sample (referred to as “initial” activity). Leaves which had a sufficient amount of active manganese (i.e., a control leaf) showed no response to the induction, while $-\text{Mn}$ leaves were restored to about 70% of the activity of the control 48 h after the induction (Figure 2B). The ratio of induced/initial activity clearly demonstrates the increase of activity in the $-\text{Mn}$ leaves as compared with the control (Figure 3). Manganese-deficient leaves were the only ones to respond positively to manganese infiltration (Figure 3), showing the specificity of this reaction.

The Hill activity rate of differentially treated lemon leaves after induction by Mn infiltration. Activity at time 0 was measured.

Figure 2. Hill activity rate of differentially treated lemon leaves after induction by Mn infiltration. Activity at time 0 was measured.

Discussion

The results demonstrate the increase in chloroplast protein and nitrogen per unit chlorophyll in manganese-deficient lemon seedlings. A chloroplast protein increase was also found in manganese-deficient algae (Kutyurin et al. 1976) and in maize grown under deficiency of some macro-nutrients (Baszynski et al. 1972).

The chloroplast protein content expressed on a chloroplast N basis increased under $-\text{Mn}$ conditions by 17% (7.18 mg chloroplast protein/mg chloroplast N in the $-\text{Mn}$ and 6.12 in the control). This increase apparently indicates a more intense protein synthesis in the Mn deficient chloroplasts.

The decrease in chlorophyll $a/b$ ratio in the $-\text{Mn}$ chloroplasts is in agreement with other reports (Bar-Akiva 1961, Spencer and Possingham 1960, Jahn and Young 1976). The relatively higher percentage of the chlorophyll $b$ in the $-\text{Mn}$ chloroplasts may result from its greater resistance to degradation (Jahn and Young 1976) or its intense synthesis under $-\text{Mn}$ conditions (Anderson and Pyliotis 1969) or both.

The chloroplasts isolated from the manganese-deficient lemon leaves had considerably reduced Hill reaction activity, as found in many other plants. Manganese infiltration into the leaves increased the Hill reaction rates in the $-\text{Mn}$ leaves up to 70% of the control leaves, after which the activity rates were not changed. The reactivation succeeded only with intact leaves or leaf fragments but not with isolated chloroplasts, confirming the results of Possingham and Spencer (1962).

The function of manganese in photosystem II is still an open question. The assumption that active manganese is
associated with protein (Radmer and Kok 1975) and the rapid restoration process (20—30 min in algae; Arnon 1958), supports the assumption that manganese is involved in some catalytic reaction in the oxygen-evolving system and that the reactivation is merely an in vivo reaction of the manganese with an existing apoprotein. In this respect the reaction resembles induction or reactivation of metallo enzymes such as nitrate reductase with molybdenum (Afridi and Hewitt 1964, Shaked and Bar-Akiva 1967), peroxidase with iron (Bar-Akiva and Lavon 1968), and ascorbic acid oxidase with copper (Bar-Akiva et al. 1969). As with these enzymes, the ratio of the Mn induced/initial Hill activity may serve as a measure for potential response to manganese application of the tested plant.


References


