Correlative Studies on Plant Growth and Metabolism. I. Changes in Protein and Soluble Nitrogen Accompanying Gibberellin-Induced Growth in Lettuce Seedlings

Vinay K. Rai and Manmohan M. Laloraya
Department of Botany, University of Allahabad, Allahabad, India

Introduction

The remarkable increase in longitudinal growth of plants by applied gibberellins is well known. Information on accompanying metabolic changes is much more limited and often concerned with relatively late stages of the growth response so that its bearing on the primary changes caused by the gibberellin is uncertain. The present paper deals with changes in protein and soluble nitrogen in gibberellin-treated lettuce seedlings. Metabolism of the nitrogenous compounds was chosen because increase in growth is generally correlated with protein synthesis. This has recently been shown for growth induced by auxin (5,8) and kinins (9). No clear-cut relationship has yet been reported with gibberellin-induced growth, although dislocation in nitrogen metabolism has been inferred by the reported loss of nicotine from gibberellin treated tobacco plants (1,10). The fact that gibberellin effects are more marked in intact plants than in isolated sections, required that these changes be studied in intact system. Lettuce seedlings were chosen as material because the response of many epigeic seedlings to gibberellin is quite rapid and because it is often limited to certain organs of the seedling while the growth of the other parts is unaffected. This permits one to establish correlations between the organ most influenced by gibberellin and the metabolic effects of the latter on other parts which are not visibly affected. In lettuce seedlings, the gibberellin response occurs mostly in the hypocotyl where as the cotyledons and roots exhibit no marked changes.

Materials and Methods

Two varieties of lettuce, Lactuca sativa var. Great Lakes and Arctic King have been used. The seedlings were grown as described earlier (4). The germinated seeds, with radicles of 1 to 2 mm, were transferred to petri dishes lined with filter paper and moistened with 6 ml of either distilled water or gibberellic acid (GA) solution. Each petri dish contained 25 to 30 seedlings and these were allowed to grow in light of 500 lux provided by daylight flou-

rescent tubes. Samples for growth measurements and analysis were taken at desired intervals. Growth as well as analytical values are averages of 3 replicates; for each replicate a sample of 50 seedlings was used. The protein and soluble-N were estimated by the routine micro-kjeldhal method, after precipitation of proteins by 10% trichloroacetic acid at 4°, as described elsewhere (9). The results have been duplicated in 2 separate experiments.

Results

GA Induced Changes at Different Growth Periods in Lettuce Seedlings Var. Great Lakes. The growth of the hypocotyl and the accompanying changes in protein and soluble nitrogen in seedlings grown in water and in 10 mg/liter GA solution are shown in figure 1.

The growth of the hypocotyl of treated seedlings exhibits a linear increase up to a period of 7 days although the changes in fresh weight are not of the same order as the changes in the length. This is due to greater internal differentiation of the hypocotyl taking place in the water-grown seedlings.

![Figure 1](https://www.plantphysiol.org)

**Fig. 1.** Growth and nitrogen changes in the hypocotyl of water control and gibberellin treated lettuce seedlings var. Great Lakes, over a period of 7 days. Figure on left, changes in length and fresh weight; figure on right, changes in protein and soluble nitrogen.

-- X -- water control, -- ○ --. The concentration of GA was 10 mg/liter.
The hypocotyls of the GA-treated seedlings also show higher protein content although the general pattern of the curve is the same as in the controls. This increase is of the order of 115 to 130% during the first 5 days when growth increase is of the order of 200% and 320% in fresh weight and length respectively.

The GA-induced growth changes, particularly of fresh weight, in hypocotyl show greatest correlation with the changes in soluble-N (fig 2). The pattern of the 2 curves is similar especially during the third to seventh day of growth. A slight decrease in soluble-N in the GA-treated seedlings during the first 2 days, as against the controls, is probably due to its being utilized in the early increase of the protein content which is marked even on the second day of seedling growth.

The increase in the protein-N and soluble-N content in the hypocotyl is accounted for by the losses of nitrogen in the cotyledons and roots of the GA-treated seedlings. This is shown in figure 2. C and D. During the first 3 days the protein content in the cotyledons falls by about 20% of the controls. This decrease is accompanied by a marked increase of soluble nitrogen content in the same organ; this being of the order of 200% of the controls on the third day. The cotyledons of the GA-treated seedlings were more expanded and this is evident by the increase in their green weight up to the 7 days of growth.

The roots of the gibberellin-treated seedlings exhibit gradual decline, up to 7 days of growth, in the fresh weight, protein-N, and soluble-N although with no apparent change in length, as compared to the controls. The increase in the soluble-N on the first day could be due to initial activated distribution of nitrogen from the cotyledons prior to any growth changes in the hypocotyl. The decline in the protein content attains a constant level on the third day which is about 65% of the control value. The soluble-N content is also less by about the same order as the protein between third and fifth days and shows further decline on the seventh day.

A comparison of the metabolic pattern of GA-treated seedling and the control, when considered as a whole presents the most interesting feature of gibberellin-induced growth. Figure 3 shows the changes in protein and soluble nitrogen in entire seedlings.

The water-grown seedlings show an initial increase in the protein content with a peak on the third day while in GA-treated seedlings the protein content declines even during the first 3 days and falls sharply to a low value on the seventh day of growth. Conversely the soluble-N content of the GA-treated seedlings increases sharply from the early period up to the seventh day of growth while in the controls the soluble-N content is low during the first 3 days and shows some increase only during the fifth and seventh day of growth when the protein curve is already declining.

Consideration of the total nitrogen changes in the GA-treated and water-control seedlings show that the total nitrogen content is not changed (table 1). It is, therefore, clear that redistribution of nitrogen accompanies GA-induced growth. Yabuta et al. (11) have also shown that the total nitrogen content does not change in gibberellin-treated rice seedlings.

**Gibberellin Concentration, Growth and Nitrogen Relationships in Lettuce Seedlings Var. Arctic King.** At all the concentrations of GA used in these experiments increase in protein content in the hypo
Fig. 3. Changes in protein and soluble nitrogen in entire seedling of lettuce var. Great Lakes. - \( \text{GA}_3 \), water control, + \( \text{GA}_3 \), 10 mg/liter of \( \text{GA}_3 \).

Table I. Showing Changes in Fresh Weight and Total Nitrogen Content in Entire Lettuce Seedlings (L. sativa var. Great Lakes)

<table>
<thead>
<tr>
<th>mg/liter ( \text{GA}_3 )</th>
<th>Days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fr wt</td>
<td></td>
<td>0</td>
<td>207.7</td>
<td>309.1</td>
<td>421.3</td>
<td>607.1</td>
</tr>
<tr>
<td>mg</td>
<td></td>
<td>10</td>
<td>211.9</td>
<td>374.0</td>
<td>532.1</td>
<td>799.0</td>
</tr>
<tr>
<td>Total N</td>
<td></td>
<td>0</td>
<td>1517.2</td>
<td>1582.3</td>
<td>1687.7</td>
<td>1673.4</td>
</tr>
<tr>
<td>mg</td>
<td></td>
<td>10</td>
<td>1491.9</td>
<td>1582.8</td>
<td>1584.7</td>
<td>1686.9</td>
</tr>
</tbody>
</table>

cotyl is of the same order namely about 30% over the controls, although the growth effects are variable at least at the concentrations of 1 mg/liter and 5 mg/liter of \( \text{GA}_3 \) (fig 4). The increase in soluble-N in this experiment is not of the same order as those in the earlier experiment. This may be due to the divergent growth effects observed in the 2 varieties of lettuce used. It has been shown in the earlier experiment that at seventh day when the observed growth effect is optimum, namely about 400% of the control, the soluble-N content drops to a value showing only 20% increase over the control. The growth effect on the fourth day in variety Arctic King is even greater than that observed at the seventh day in variety Great Lakes. The relative low increase of soluble-N on the fourth day in the former variety may thus be imposed due to the early decline of the soluble-N content because of the very pronounced growth in shorter period.

The changes in the cotyledons and roots corroborate the earlier results that during gibberellin-induced growth protein is depleted in the cotyledons and the soluble-N, which so accumulates, is translocated to the growing hypocotyl. The lower protein and soluble-N content in the roots of the treated seedlings also suggest that most of the nitrogenous matter is withheld in the hypocotyl to sustain the growth effects of gibberellin.

**Discussion**

The data clearly indicate that gibberellin-induced growth of the hypocotyl is accompanied by an enhanced mobilization of the nitrogen reserves from
the cotyledons. This is reflected by a much faster rate of loss of protein nitrogen from the cotyledons of the GA-treated seedlings than in the controls. The data may be compared to those of Paleg et al. (6) who showed increased mobilization of protein nitrogen and reducing sugars released from the barley endosperm tissue treated with gibberellin, and of Halevy et al. (3) who have shown loss of dry weight of the cotyledons in gibberellin-treated cucumber seedlings. The latter workers also pointed out that reduction in the dry weight of the cotyledons was caused mainly by translocation to the hypocotyl. The increase in protein and soluble-N content, as found in the hypocotyl of the GA-treated lettuce seedlings, thus lends support and extends the work of Halevy et al. It will be observed, however, that no direct relationship could be established between the growth effects and loss of protein-N from the cotyledons in the concentration range of the GA studied in the present investigation. The protein losses from the cotyledons at all the 3 concentrations of GA are of about the same order while the growth effects are different. Similarly the protein gain in the hypocotyl is of the same order at all the concentrations of GA. Thus, increase in protein is also not correlated with the growth effects. Whether this is due to the higher concentration ranges used in these experiments, than in the work of Paleg et al. (6) who measured the activity over a wide range of lower concentrations, is not yet clear and is under study. It is of some interest in this connection to refer to the work of Halevy et al. (3) who showed that although the growth effects of 4 gibberellins, GA1, GA2, GA3, and GA4 in the cucumber hypocotyl system were quite different, the translocation of cotyledonary reserves to the hypocotyl was of the same order.

The nitrogen losses in the root, at different concentrations of GA, is of the same order as the nitrogen gain in the hypocotyl. It would appear that the GA-induced growth effects are accompanied by an altered distribution of nitrogen in different parts of the seedling so that more is retained in the hypocotyl than translocated to the roots. The growth increase in hypocotyl, therefore, shows greatest correlation with the changes in soluble-N. Similar redistribution of ribonucleic acid content, accompanying GA-induced growth has been shown earlier (7).

Somewhat similar conclusions have been reached by Halevy et al. (3) who have shown that increased activity is due not to an increase in translocation of dry matter from the cotyledons but to internal distribution of more dry matter to the hypocotyl rather than to the radicle. The dry matter increase in the hypocotyl of the GA-treated seedlings could be accounted for by increased translocation and retention of nitrogen in this organ.

While the hypocotyls of GA-treated seedlings show an increase in the protein content on a per hypocotyl basis, calculations based on protein content per unit fresh weight present a very different picture. The protein content per unit fresh weight is only about 50% of the control, and per mm length it is even less, namely about 25% of the control. Thus, increase in protein content does not keep pace with the longitudinal growth and fresh weight changes which are so markedly influenced by gibberellin. The increased fresh weight of the seedling is due largely to increased water content in the hypocotyl although some increase occurs also in the cotyledonal leaves which expand with gibberellin treatment.

The protein/soluble-N ratio in gibberellin-treated seedlings is very much lower than the control seedlings. The metabolic pattern of the GA-treated seedlings reminds one of the metabolic patterns of the dark grown seedlings. Whether this apparent similarity is superficial and a consequence of GA effects or represents the controlling mechanism of the growth effects of GA is not yet clear. It may be of some interest to note that gibberellin-induced increase in fresh weight of the seedling takes place when protein content is in fact decreasing. Enhanced synthesis of protein during auxin-induced increase in fresh weight has been reported (3,8) and this has been linked with the mechanism of water uptake. The results presented here do not favour the view that enhanced synthesis of protein is essential for water uptake.

Summary

Changes in protein and soluble-N content accompanying gibberellin-induced growth of the hypocotyl in lettuce seedlings have been studied. It has been shown that GA treatment results in enhanced mobilization of reserve nitrogen from the cotyledons to the growing axes. More of the nitrogen is, however, retained in the hypocotyl than is translocated to the roots. The internal distribution of the translocatable nitrogen is thus altered by gibberellin treatment.

The growth effects of GA show no direct relationship either with the loss of protein from the cotyledons or the extent of enhanced protein synthesis in the hypocotyl. The change in the soluble-N content in different parts, however, shows close correlation with growth effects.

The gibberellin-treated seedlings exhibit a lower protein/soluble-N ratio than the water-grown seedlings. The increase in fresh weight is in fact accompanied by the loss of protein. This is against the view that increased protein synthesis and water uptake go hand in hand during growth.

Acknowledgment

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Isolation and Properties of Gibberellin-like Substances from Citrus Fruits 1, 2

Rashad A. Khalifah, Lowell N. Lewis, and Charles W. Coggins, Jr.
University of California Citrus Research Center, Riverside, California

The first direct evidence for the occurrence of gibberellin-like materials in higher plants was reported by Mitchell et al. in 1951 (4). Since then, the occurrence of native gibberellins in different families of flowering plants has been reported (5, 6, 7, 8). The isolation of gibberellin A1 from the water sprouts of mandarin orange (Citrus unshiu) was reported by Sumiki and Kawarada (9). To our knowledge, this is the only published report concerned with identification of naturally occurring gibberellins in a citrus tissue.

The present investigation stems from work on fluorometric and biological characterization of naturally occurring hormones in citrus fruits. Evidence is presented for the natural occurrence of 3 different gibberellin-like substances, 2 of which behaved similarly to gibberellins A1 and A0, in orange and lemon fruits.

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Materials and Methods

Young fruits of Washington Navel oranges, Citrus sinensis (Linn.) Osbeck, and Eureka lemons, Citrus limon (Linn.) Burmann, ranging in size between 3.0 mm and 21.0 mm diameter were harvested for analysis over a period of 5 months. Samples were picked from healthy trees grown under normal field conditions at the Citrus Research Center of the University of California, Riverside. Immediately after harvest, the fruit was frozen in dry ice, ground, and lyophilized. The extraction procedure was a modification of the method described by Sumiki and Kawarada (9). Diethyl ether was the organic solvent used and the final extract was fractionated by column, paper, and/or thin-layer chromatography.

In column chromatography, a mixture of silicic acid (20 g) and Celite 535 (2 g) was used. The mixture was slurried with chloroform and then poured into a 2.5 x 34 cm column. Elution was carried out with ethyl acetate (150 ml) : chloroform (150 ml) followed by 100% isopropanol (fluorometric grade). An automatic fraction collector was used to obtain 5 ml fractions of the column eluates,

Literature Cited