Correlative Studies on Plant Growth and Metabolism II.
Effect of Light and of Gibberellic Acid on the Changes in Protein and Soluble Nitrogen in Lettuce Seedlings

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Summary. Protein and soluble nitrogen distribution in different parts of lettuce seedling was studied in light and darkness and in presence and absence of gibberellic acid. In dark, applied gibberellic acid failed to show any marked effect on the nitrogen changes in lettuce. Light inhibits translocation of nitrogen reserves from the cotyledons. Gibberellic acid reverses the light inhibition of longitudinal growth but has no effect on the inhibition of translocation from the cotyledons. Light grown, gibberellic acid treated seedlings exhibit a pattern of protein and soluble-N which is characteristic of the dark grown seedlings. Thus gibberellic acid not only causes morphological reversal of light inhibition but also shifts the nitrogen metabolism of light grown plants, close to that of plants grown in darkness.

Materials and Methods

Lactuca sativa var. Great Lakes, used in the present experiments were obtained from Messrs. P. Poacha & Sons, Poona. Seeds when soaked in complete darkness showed about 50% germination after about 36 hours of soaking. Germinated seeds with 1 to 2 mm radicle emerged, were transferred to petri-dishes lined with filter paper and moistened with either 6 ml of distilled water or gibberellic acid (GA) solution. During manipulations a green safe light was used. Seedlings were allowed to grow in complete darkness at a temperature of 25 ± 1°C. Samples for growth measurements and analysis were taken at desired intervals. Growth as well as analytical values are average of 3 replicates, for each replicate a group of 50 seedlings was used. The protein and soluble nitrogen were determined by micro-Kjeldahl method, following precipitation of protein by 10% (W/V) trichloroacetic acid at 4°C, as described elsewhere (15).

Results

Effect of GA on Growth and Nitrogen Changes in Lettuce Seedlings Grown in Darkness. Though originally it was shown that gibberellins do not show any growth response on plants grown in complete darkness, recently certain plants have been shown to exhibit some stimulation of growth even in darkness (8). The observed growth response was, however, very little as compared to that in light. The lettuce var. Great Lakes used in the present study, however, also showed some
When the total protein-N and soluble-N content in the entire seedlings of both the water controls and GA-treated ones, is considered, it is noted that unlike the seedlings grown in light (13), here there is no marked change in the pattern of protein-N and soluble-N changes due to GA application throughout the growth period studied (table I).

**Fig. 1.** Showing the pattern of growth and nitrogen distribution in different parts of dark grown GA-treated seedlings of *Lactuca sativa* var. Great Lakes. A) Changes in length (— □ — ) and fresh weight; B, C, and D) Changes in protein and soluble nitrogen in hypocotyl (B), roots (C), and cotyledons (D). — x — Fresh weight, — ● — protein-N, — O — soluble-N.

growth response in length and in fresh weight even when germinated and grown in complete darkness (fig 1).

The results of a typical experiment are presented in figure 1. Though grown in darkness some increase is indeed noted in the length of the treated hypocotyl which is also corroborated by similar increase in fresh weight of the same organ. As in the case of GA treated light grown seedlings (13), here too roots show a decrease in fresh weight without any apparent effect on length. The cotyledons also record slightly higher fresh weight in GA-treated seedlings, showing value of about 25% above water control.

Changes in protein-N and soluble-N in hypocotyl show that although some increase in protein content is observed on the second day of growth, the values reach close to the control on the third and fifth day. However, again on the seventh day, there is a definite increase in protein nitrogen in GA-treated sets.

The soluble-N content does not show any marked changes from the control. The pattern in roots is very much similar to that obtained in the experiment in light (13). The curves for fresh weight, protein-N and soluble-N show close similarity. A decrease in nitrogen content up to third day of growth, and then its recovery during fifth and seventh day is apparent. The cotyledons though do not show any increase in size but show significant increase in fresh weight and soluble-N content; both the curves show close similarity in pattern.

The 7 days of growth studied (fig 2). The inhibition of translocation from the cotyledons in light, is reflected in the lower values of nitrogen for hypocotyl. With growth period the nitrogen value of hypocotyl in light, keeps almost a constant value while the same in dark shows a progressive increase during whole experimental period. The changes in roots are, however, not so marked and little higher values are recorded from second day onwards.

Although GA causes the light grown seedlings to resemble those grown in dark and changes the nitrogen metabolism of light grown seedlings close to one grown in the dark (as shown under a subsequent heading), it has no effect on the inhibition of nitrogen translocation from the cotyledons, caused by light.

Table II shows that in seedlings grown in light and treated with GA, the nitrogen of the cotyledons do not show any appreciable change due to GA treatment. It may be observed that while in dark the nitrogen content of the cotyledons falls sharply (fig 2) in GA-treated light grown seedlings, the nitrogen values of cotyledons show no significant change (table II). The higher values of nitrogen
Table III. Growth and Nitrogen Value of Lactuca sativa var. Arctic King Seedlings Grown in Light, as Altered by Gibberellin Concentration

<table>
<thead>
<tr>
<th>Plant part</th>
<th>0</th>
<th>1</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>138.4</td>
<td>114.4</td>
<td>114.4</td>
<td>121.2</td>
</tr>
<tr>
<td>Hypocotyl*</td>
<td>76.3</td>
<td>202.1</td>
<td>225.5</td>
<td>217.1</td>
</tr>
<tr>
<td>Cotyledons</td>
<td>132.8</td>
<td>193.9</td>
<td>212.8</td>
<td>220.8</td>
</tr>
</tbody>
</table>

The data clearly indicate that with increasing concentration of gibberellin, though there is a progressive increase in length and fresh weight of the hypocotyl, fresh weight of the cotyledons and decrease in fresh weight of roots, the nitrogen distribution in different parts is not much affected by change in GA concentration.

Changes in Nitrogen Pattern of Seedlings Grown in Light and Darkness in Presence and Absence of Gibberellic Acid. To pinpoint the similarity of pattern of nitrogen metabolism between dark grown seedlings and light grown GA treated seedlings data were analysed for the changes in protein-N and soluble-N in entire lettuce seedlings grown in light and darkness, with and without GA (fig 3).

Figure 3-A shows pattern of protein and soluble-N changes in light and dark grown seedlings, grown on distilled water alone. If we compare the nitrogen pattern of dark grown seedlings with

Table II. Effect of Gibberellin on Distribution of Nitrogen in Different Parts of Lactuca sativa

These plants were grown in light.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant part</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>Total Nitrogen in µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Roots</td>
<td>148.0</td>
<td>250.9</td>
<td>367.3</td>
<td>369.2</td>
<td>282.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypocotyls</td>
<td>151.2</td>
<td>165.7</td>
<td>165.4</td>
<td>153.3</td>
<td>127.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cotyledons</td>
<td>1218.0</td>
<td>1165.7</td>
<td>1150.8</td>
<td>1110.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA 10 mg/l</td>
<td>Roots</td>
<td>144.9</td>
<td>225.8</td>
<td>243.5</td>
<td>241.6</td>
<td>165.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypocotyls</td>
<td>147.2</td>
<td>187.9</td>
<td>204.1</td>
<td>227.8</td>
<td>188.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cotyledons</td>
<td>1199.9</td>
<td>1169.1</td>
<td>1137.2</td>
<td>1217.5</td>
<td>1208.0</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 3. Showing changes in protein and soluble-N pattern of entire Lactuca sativa var. Great Lakes, seedlings over a period of seven days. A) Grown in light (L) and in darkness (D), B) grown in darkness in presence (→□→) and absence (→○→) of gibberellic acid. Open symbol soluble-N, closed symbol protein-N.

Discussion

In our previous communication (13) we have shown that promotion of lettuce hypocotyl growth in light, by application of GA, is accompanied by an increased content of protein-N and soluble-N in the hypocotyl. This increase is reflected directly into the lower values for both protein-N and soluble-N in the roots, which also shows a decline in fresh weight due to GA-treatment.

An analysis of nitrogen metabolism of dark grown lettuce seedlings show that in dark, protein

the light grown, GA-treated seedlings, the 2 curves are found to be identical (compare with fig 3 of 13).

That GA treated dark-grown seedlings show the absence of the hump obtained on the third day of seedling growth even in dark, and decline in protein-N content assumes a steeper curve, is clear from figure 3-B. The soluble-N content also shows slightly higher values than water control. This may be correlated with the little growth promotion that is observed even in complete darkness in this particular lettuce variety.
or soluble-N is not markedly influenced by GA-treatment. The little changes observed, do strengthen the results obtained with light grown seedlings; and may be correlated with the little observed growth promotion even in darkness. The little growth promotion in darkness may be the result of releasing of the inhibition caused by the endogenous inhibitors present in the seed. Kollar (1962) has shown that germination inhibition of lettuce seeds is controlled by the photoperiodic treatment which the plant received during fruit setting and ripening.

It is well established that light inhibits the longitudinal growth of plants and that this inhibition is associated with changes in the pattern of nitrogen metabolism. Halevy et al. (3), have shown that light inhibits the translocation of dry matter from dark grown cucumber seedlings. This has been confirmed in our experiments with lettuce, for the translocation of nitrogen from the cotyledons. While gibberellic acid is able to reverse the inhibition of longitudinal growth caused by light, it shows no effect on the translocation of nitrogen from the cotyledons caused by light. It is further noted that though with increasing concentration of gibberellin the hypocotyl shows a progressive increase in length, it has no effect on the translocation of nitrogen from the cotyledons caused by light. The results clearly demonstrate that gibberellin effects on seedling growth are not only superficially akin to growth under etiolation conditions but also that the 2 exhibit striking similarity in their metabolic pattern. The similarities between GA induced growth and dark seedling growth are at least suggestive that growth during etiolation may be under the dominant control of gibberellin and the 'inhibitor' produced in light must be able to switch over the metabolic pattern in such a manner so as to support the differentiated growth like the thickening of the hypocotyl and expansion of the cotyledons following the inhibition of longitudinal growth in light.

**Literature Cited**