NET ASSIMILATION RATE AND GROWTH BEHAVIOR OF BEANS AS AFFECTED BY GIBBERELLIC ACID UREA AND SUGAR SPRAYS

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Many workers have demonstrated the remarkable effect of gibberellins in producing increased rates of growth in size and weight. Weight increases usually are a result of higher photosynthetic activity, but a recent study with radioactive carbon (2) indicates that gibberellic acid did not enhance the rate of CO₂ fixation per unit of leaf tissue and did not alter the general pathway of the newly fixed carbon in the sugars, organic acid, and amino acid products.

The main purpose of the present study was to determine the influence of gibberellic acid on the net assimilation rate of a plant, as measured by the technique more commonly used by the English physiologists and lately reviewed by Watson (3). It is believed that this simple technique gives more reliable information on the average rate of photosynthesis than do those involving gas exchange measurements in which the plants or leaves are placed in an artificial environment. Sugar and urea sprays were included in the experiment in an attempt to determine whether or not carbohydrates and nitrogen might become limiting factors for growth when gibberellic acid is applied.

MATERIALS AND METHODS

Matched pairs of two-week-old red kidney bean seedlings (Phaseolus vulgaris) carefully selected for uniformity in size and leaf area and grown in Hoagland's nutrient solution, were placed in forty-eight 500 ml amber bottles, in eight groups of six, corresponding to the following treatments: 1) Control (no treatment). 2) Sucrose spray (10 % solution). 3) Urea spray (2 % solution). 4) Gibberellic acid spray (50 ppm solution). 5) Sucrose plus urea (10 % and 2 %, respectively). 6) Sucrose plus gibberellic acid (10 % and 50 ppm, respectively). 7) Urea plus gibberellic acid (2 % and 50 ppm, respectively). 8) Sucrose plus urea plus gibberellic acid (10 %, 2 % and 50 ppm, respectively).

Before the treatments were applied one seedling of each pair, taken at random, was used for dry weight and leaf area measurements. The remaining plants were then treated with the chemicals and distributed at random on the greenhouse bench. Sugar and urea sprays were repeated on the third and sixth day after the initial application. The plants were harvested on the tenth day for computing final dry weight (leaves, stem, roots, and total), plant height, and leaf area. Net assimilation rate (in grams per square decimeter of leaf surface and per day) was calculated by the formula (3).

\[ \text{NAR} = \frac{(W_2-W_1) \ (\log L_2-\log L_1)}{(L_2-L_1) \ (t_2-t_1)} \]

where \(W_1\) and \(W_2\) are total dry weight and \(L_1\) and \(L_2\) total leaf area at times \(t_1\) and \(t_2\), respectively. The relative growth rate (in grams per gram per day) was given by:

\[ \text{RGR} = \frac{(\log W_2-\log W_1)}{(t_2-t_1)} \]

The data were analyzed for main effects and interactions of second and third order between gibberellic acid, sugar and urea, as a 2² factorial experiment.

RESULTS AND CONCLUSIONS

From the results presented in table I, which gives also the F values of statistical analysis, the following conclusions are derived:

1) Net assimilation rate (NAR) and relative growth rate (RGR) were both increased by gibberellic acid; in other words, the amount of dry matter produced by photosynthesis per unit of leaf area was significantly higher in plants receiving gibberellic acid. On a percentage basis, the average increase was 17.1 % for NAR (0.041 and 0.035 g/dm²/day,
Fig. 1. Influence on the growth of red kidney bean plants of the treatments indicated. For explanation see text.
Table I
Effect of Gibberellic Acid, Sugar, and Urea Sprays on Net Assimilation Rate (NAR), Relative Growth Rate (RGR), Height, Leaf Area, and Dry Weight of Beans

<table>
<thead>
<tr>
<th>Treatments</th>
<th>NAR (g/dm²/d)</th>
<th>RGR (g/g/d)</th>
<th>Height (cm)</th>
<th>Leaf Area (dm²)</th>
<th>Dry Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check</td>
<td>0.035</td>
<td>0.097</td>
<td>32.1</td>
<td>3.53</td>
<td>1.25</td>
</tr>
<tr>
<td>Sugar</td>
<td>0.038</td>
<td>0.098</td>
<td>32.6</td>
<td>3.31</td>
<td>1.36</td>
</tr>
<tr>
<td>Urea</td>
<td>0.029</td>
<td>0.074</td>
<td>28.9</td>
<td>2.89</td>
<td>1.07</td>
</tr>
<tr>
<td>Sugar + Urea</td>
<td>0.039</td>
<td>0.098</td>
<td>31.2</td>
<td>3.74</td>
<td>1.25</td>
</tr>
<tr>
<td>Gibb. Acid</td>
<td>0.038</td>
<td>0.104</td>
<td>72.3</td>
<td>3.47</td>
<td>1.35</td>
</tr>
<tr>
<td>Gibb. + Sugar</td>
<td>0.042</td>
<td>0.112</td>
<td>69.9</td>
<td>3.99</td>
<td>1.52</td>
</tr>
<tr>
<td>Gibb. + Urea</td>
<td>0.039</td>
<td>0.107</td>
<td>79.5</td>
<td>3.85</td>
<td>1.45</td>
</tr>
<tr>
<td>Gibb. + Sugar + Urea</td>
<td>0.044</td>
<td>0.111</td>
<td>79.6</td>
<td>4.21</td>
<td>1.66</td>
</tr>
</tbody>
</table>

Sources of variation

| Effect of Gibb. acid | 13.91*** | 16.76*** | 892.3*** | 7.06** | 12.07*** | 6.68** | 2.93 | 77.22*** |
| Effect of Sugar      | 12.13*** | 5.06**   | 0.09     | 3.76   | 10.88*** | 5.01** | 16.00*** | 2.22 |
| Effect of Urea        | 0.13     | 1.48     | 4.41**   | 0.25   | 0.17     | 3.09   | 0.57 | 1.45     |
| Interaction Gibb. x Sugar | 0.49   | 0.49     | 0.73     | 0.10   | 0.07     | 1.33   | 0.01 | 0.41     |
| Interaction Gibb. x Urea | 2.06   | 2.36     | 13.12*** | 1.10   | 2.17     | 3.88   | 0.55 | 2.94     |
| Interaction Sugar x Urea | 1.92   | 1.16     | 0.55     | 1.38   | 1.25     | 2.18   | 0.13 | 2.52     |
| Inter. Gibb. x Sugar x Urea | 0.22 | 2.94     | 0.10     | 2.80   | 0.62     | 2.15   | 1.77 | 2.00     |

* Includes the petioles
** Significant (5 % level)
*** Highly significant (1 % level)

For treated and untreated plants, respectively, and 17.4 % for RGR (0.108 and 0.092 g/g/day for treated and untreated plants, respectively). Sugar also seemed to have increased NAR and RGR but this was obviously a result of the increase in dry weight brought about by the weight of the added sugar itself. Urea spray when applied alone caused necrotic spots on the leaves and decreased NAR and RGR, but in combination with sugar it seemed to stimulate NAR, although this effect was not statistically significant.

2) Plant height, as expected, showed the most striking response to gibberellic acid, with an average increase of 141.3 % (75.3 and 31.2 cm for treated and untreated plants, respectively). Urea sprays also decreased plant height when applied alone but had a stimulative effect when applied with gibberellic acid (positive interaction). This indicates that nitrogen might become a limiting factor for stem elongation when gibberellic acid is applied. Sugar sprays had no significant effect on plant height.

3) Leaf area increased 151.1 % by gibberellic acid (3.88 and 3.37 dm² for treated and untreated plants, respectively). Sugar sprays also seemed to have increased leaf area but this effect was not statistically significant. Urea sprays also reduced leaf area when applied alone (a 2 % solution, as used, is generally considered too strong for herbaceous plants), but not in combination with either sugar or gibberellic acid. Necrotic spots were not observed in plants treated with urea combined with either sugar or gibberellic acid, thus indicating that these substances are equally effective in preventing urea toxicity. Sugar sprays have been reported to have such an effect in other plants (1).

4) Total dry weight also was increased by gibberellic acid (17.3 %) and by sugar (16.4 %). Attention is called to the fact that the increase induced by the former took place in the stem whereas sugar sprays affected primarily leaf and, to a less extent, root dry weight. Gibberellic acid reduced root dry weight (17.9 %), a response that was effectively counteracted by sugar sprays (15.3 % increase). Urea sprays showed no significant influence on dry weight.

The above findings clearly indicate that gibberellic acid (probably as a consequence of its market influence on stem elongation) accelerated mobilization of photosynthates from the leaves to the stem. Higher NAR in plants receiving gibberellic acid probably resulted from this more rapid removal of photosynthates from the leaves. Carbohydrate supply limited root growth when gibberellic acid was applied, due to competition by the stem.

The above results do not support Haber and Tolbert's findings (1) according to which gibberellic acid does not affect the rate of photosynthesis. These authors worked with detached leaves, where mobilization of photosynthates is impeded and photosynthesis is bound to be retarded by the accumulation of its own products. Inasmuch as increases in NAR as induced by gibberellic acid seem to result from speeded translocation of photosynthates to the stem, results with detached leaves, in the author's opinion, are not applicable to intact plants.

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Summary

Gibberellic acid increased net assimilation rate, relative growth rate, stem dry weight, leaf area, and plant height. Root dry weight was reduced and leaf dry weight was not significantly altered. Reduction in root dry weight induced by gibberellic acid could be effectively controlled by 10% sucrose sprays. Increase in net assimilation rate (photosynthesis) caused by gibberellic acid seemed to result from a more rapid translocation of photosynthates from the leaves to the stem. Urea sprays increased stem elongation of plants receiving gibberellic acid. Both sugar and gibberellic acid were effective in protecting against injury by 2% urea spray.

Literature Cited

Conditions Affecting Enzyme Synthesis in Cotyledons of Germinating Seeds 1, 2

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The rapid and manyfold increase in enzymatic activities of a phosphatase and an amylase in the cotyledons of germinating peas has been reported to be the result of net enzyme synthesis (8, 9). This conclusion was based upon the following findings:

I. Increases in these enzymatic activities are prevented by the protein synthesis inhibitors, chloramphenicol, and p-fluorophenylalanine.

II. Active mitochondria can be isolated from germinating pea cotyledons through the tenth day of germination. There is, therefore, presumably sufficient energy available for protein synthesis.

III. The microsomal fraction of pea cotyledons is capable of an in vivo and in vitro incorporation of glycine-C-14 into protein through the tenth day of germination.

IV. It has been shown in vitro experiments that the microsomal fraction isolated from germinating peas is capable of a net synthesis of soluble proteins (7).

Further experimentation has shown that development of these enzymatic activities is dependent upon presence of the axis tissue (10). When the axis tissue is dissected away from the cotyledons of the dry seeds and the cotyledons placed under germination conditions, there is no development of phosphatase. These excised cotyledons rapidly lose mitochondrial activity; supposedly the loss of mitochondrial function is responsible for lack of enzyme synthesis in the excised cotyledons. Development of phosphatase activity in the cotyledons of germinating pea seeds is also greatly affected by temperature. At 16°C, although there is germination and slow growth in the seedling, there is no increase in the phosphatase activity of the cotyledons. During early stages of germination there is a rapid production in the axis tissue of a phosphatase apparently identical to the cotyledons phosphatase. Patterns of phosphatase activity in the cotyledons of germinating wax beans and the endosperm of castor beans are entirely different from the pattern in germinating peas.

Materials and Methods

Seeds were germinated in moist Vermiculite at temperatures indicated in each experiment. After the germination period the seedlings were washed free of Vermiculite and the different parts of the seedling separately homogenized in Tris buffer (0.1 M, pH 7.3) at 0°C with a Servall Omnimix. Fibrous material was removed by straining through cheese cloth. This extract was centrifuged at 20,000 × G for 1 hour and the supernatant fraction analyzed. Protein was determined by the method of Warburg and Christian (5). Phosphatase activity was mea-