Promotion of Cress Root Elongation in White Light by 3,5-Diiodo-4-hydroxybenzoic Acid

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Summary. The effect of low concentrations of 3,5-diiodo-4-hydroxybenzoic acid (DIHB) in promoting the elongation of light-exposed cress (Lepidium sativum L.) roots has been further examined. Aeration of the DIHB solution in which the roots were grown largely removed the growth promotion. The addition of ethylene or the ethylene precursor methionine to the solution caused marked inhibition of root elongation and this effect was relieved by DIHB.

Introduction

White light inhibits the rate of root elongation in a variety of plant species (Torrey, 1952; Pilet and Went, 1956; Burström, 1960; Masuda, 1962; Ohno and Fujiwara, 1967; H. Wilkins et al., 1974a, b). Recent investigations have demonstrated that the root cap of maize seedlings is solely responsible for the perception of light and that it responds to this stimulus by the production of growth-inhibiting factor(s) (H. Wilkins et al., 1974a, b; H. Wilkins and Wain, 1974). There is now evidence that abscisic acid is a growth-inhibitor produced in response to light (H. Wilkins and Wain, 1974; Tietz, 1974).

Hydrolysis of the selective herbicide 3,5-diiodo-4-hydroxybenzonitrile (Ioxynil) yields 3,5-diiodo-4-hydroxybenzoic acid (DIHB), a chemical which counteracts the inhibiting effect of white light on cress, rice and wheat root elongation (Wain et al., 1968a, b; H. Wilkins et al., 1974b). The chemical enhances cell elongation rather than cell multiplication but has little influence on the light-sensitive processes taking place in the root cap (Wain et al., 1968; H. Wilkins et al., 1974a). Maximum response to DIHB occurs when cress roots are entirely immersed in a nutrient-enriched solution rather than when the solution is applied to filter paper on which the seedlings are grown (Wain et al., 1968a, b; H. Wilkins et al., 1974a). It was decided therefore, to examine whether the method of supplying DIHB to the roots could influence the activity of the compound in promoting root elongation in the light. The possible involvement of ethylene as a factor operating in the inhibition of root growth and the removal of this inhibition by DIHB is examined in the present study and also forms the basis of a number of separate investigations proceeding in this laboratory.

Materials and Methods

The following methods were used to determine the elongation of the roots of cress seedlings (Lepidium sativum L. cv. Curled) during incubation for 64, 85 or 96 h in white light (Mazda daylight fluorescent tube, 72.52 W m⁻²) at 23 ± 1°C in contact with water or a 5 μM solution of the di-sodium salt of DIHB:

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(a) Cress seedlings were grown on Whatman No. 1 filter paper in 9 cm petri dishes containing 3.0 or 7.0 ml water or DIHB solution. This gave depths of 0.1 mm and 2.0 mm respectively of liquid lying free above the filter paper.

(b) Cress seeds were germinated on rafts consisting of nylon net covering washed polystyrene beads floating on 200 ml water or DIHB solution (30 mm deep) in covered crystallising dishes (Wain et al., 1968a; Taylor and Knight, 1968). This method is referred to as the raft test. Root elongation was assessed after incubation periods during which (a) the solutions were aerated or remained non-aerated or (b) ethylene was bubbled through the solutions. The gas flow rate was 600 ml h\(^{-1}\) in both cases and bubbling was carried out for 30 min at the beginning of the experiment and for 30 min every 12 h thereafter. In other experiments the effect of 0.1 μM DL-methionine (BDH Chemicals Ltd.) on root elongation was also established.

During the raft test the initial pH of the aqueous solutions was 4.5–4.8 and little change was detected during the experiments.

**Results and Discussion**

The activity of DIHB in promoting the elongation of roots exposed to white light is well established, but the conditions necessary for maximum activity have not been precisely defined (Wain et al., 1968a, b; H. Wilkins et al., 1974b). The present investigations have revealed that promotion of cress root elongation is related to the volume and depth of the DIHB solution in which the roots are grown. For example, 3 ml of a 5 μM DIHB solution (0.1 mm deep) has no significant effect on root elongation in the light whereas 7 ml of solution (2 mm deep) produces a 55% enhancement of elongation under the same conditions (Table 1). Since root elongation is not enhanced by DIHB in the dark (see also Wain et al., 1968a, b) all further experiments were carried out in white light only.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root length (mm)</th>
<th>Light</th>
<th>Dark</th>
</tr>
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<tbody>
<tr>
<td>3.0 ml water</td>
<td>16.40 ± 1.89</td>
<td>24.27 ± 1.72</td>
<td></td>
</tr>
<tr>
<td>7.0 ml water</td>
<td>16.80 ± 1.36</td>
<td>23.17 ± 2.00</td>
<td></td>
</tr>
<tr>
<td>3.0 ml DIHB</td>
<td>14.18 ± 1.62</td>
<td>20.00 ± 2.63</td>
<td></td>
</tr>
<tr>
<td>7.0 ml DIHB</td>
<td>26.00 ± 1.76</td>
<td>17.20 ± 2.36</td>
<td></td>
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</tbody>
</table>

Table 1. Root length 85 h after germination of cress seeds in white light or darkness at 23°C in petri dishes containing 3.0 or 7.0 ml water or 5 μM DIHB solution. Means and standard errors of 32 measurements are shown.

When cress seedlings were grown in white light on rafts with their roots immersed in 200 ml 5 μM DIHB solution in absence of nutrients, root elongation was enhanced by 80% (Table 2, treatments a, b). Aeration of the solutions used in the raft tests, however, resulted in a decrease in activity such that only a 14% promotion of elongation occurred (Table 2, treatments c, d). It would therefore appear that non-aerated waterlogged conditions operate to increase the root growth-promoting activity of DIHB in the light.

A waterlogged environment can have several physiological effects on the endogenous growth-regulator status of roots (Phillips, 1964; Burrows and Carr, 1969), one of which is an increase in ethylene production (Kawase, 1972, 1974). The presence of this compound can give rise to a thickening and shortening of the roots and to a proliferation of root hairs (Harvey and Rose, 1915; Smith and Russell, 1969). These effects are closely similar to those found in cress roots growing in water and exposed to light in the raft test. Light is known to stimulate the non-enzymatic, flavine mononucleotide-mediated, production of ethylene...