Short Communication

The Effect of Growth in 99.8% Deuterium Oxide on the Ultrastructure of Winter Rye

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ABSTRACT

A preliminary report dealing with the ultrastructural effects of culture in a 99.8% D$_2$O (deuterium oxide) environment on winter rye (Secale cereale L. cv. Winter) is presented. In general, the cells of D$_2$O-cultured seedlings appeared similar to the cells of H$_2$O-cultured seedlings. However, differences were found in chloroplast and dictyosome morphology, and ribosome number.

The biological effects of D$_2$O have been the subject of many investigations since Urey et al. (10) first discovered the compound in 1932. The vast majority of the early studies involved only a cursory recording of the changes that could be observed when the test organisms were placed in a D$_2$O-enriched solution (2). The general conclusion reached through these experiments was that high concentrations of D$_2$O are generally toxic to biological systems, however, the mechanism of this toxicity remained unclear.

Following the successful culturing of several species of green algae in essentially 100% D$_2$O by Chorney et al. (1), the question of D$_2$O toxicity and its mechanism was extensively studied by Katz et al. (6, 7) and several other investigators (1-4). This work suggested that the effects of D$_2$O on biological systems could be explained by the kinetic isotope theory. This theory, however, cannot account for the high toxicity of D$_2$O to higher life forms. Seed plants for example cannot, in general, tolerate concentrations of D$_2$O greater than approximately 70% (2). In 1964, however, Siegel and his co-workers (9) reported that seeds of the grass winter rye were able to germinate and grow for a limited time in 99.5% D$_2$O.

This paper is a preliminary report of an ultrastructural examination of D$_2$O-cultured winter rye that attempts to find some explanation for the high toxicity of D$_2$O to higher plants or give some indication as to why winter rye is at least partially resistant to this toxicity.

METHODS AND MATERIALS

Winter rye (Secale cereale L. cv. Winter) seeds were surface-sterilized and then placed in sealed Petri dishes and allowed to germinate in either sterile H$_2$O or 99.8% D$_2$O. The plants were harvested at a time of morphological similarity between the two types of plants, approximately 2 days for the H$_2$O control and 9 days for the D$_2$O-cultured plants.

For electron microscopic study, the plant tissue was fixed in Karnovsky’s fixative (5), postfixed in osmium tetroxide, dehydrated with ethanol, treated with propylene oxide, and embedded in epoxy resin. Thin sections were cut with a diamond knife on a Porter-Blum MT-2B ultramicrotome and placed on copper grids. Sections were double stained with uranyl acetate followed by lead citrate (8). The sections were viewed and photographed with a Hitachi HS-8-1 electron microscope operated at 50 kv.

RESULTS AND DISCUSSION

Winter rye seedlings cultured in 99.8% D$_2$O for 9 days appear to be at the same developmental stage as plants grown in H$_2$O for approximately 2 days. These plants have a well formed root and a mature coleoptile which encloses the first leaf and are similar in gross morphology (Table I). Root growth, however, is inhibited in the D$_2$O-cultured plants.

The similarity between plants cultured in H$_2$O and D$_2$O is also evident at the ultrastructural level (Fig. 1, A [H$_2$O] and B [D$_2$O]). Unlike the microorganisms grown in D$_2$O which have been reported to exhibit a “less well organized pattern (of growth and development) than do ordinary microorganisms” (2), the cells of D$_2$O-cultured winter rye appear similar to cells of H$_2$O-control plants. It had been expected, in light of the results with microorganisms, that some disruption at the ultrastructural level would be found. This, however, has not proven to be the case.

The lack of any major difference in ultrastructural detail between the two types of tissue is puzzling. Indeed, except for some minor, although not necessarily insignificant differences (e.g. dictyosome morphology, ribosome number, and chloroplast structure), the deuterated cells seem normal. The functionality of these cells is suggested by the presence of starch.

<table>
<thead>
<tr>
<th>Morphological Comparison of H$_2$O- and D$_2$O-grown Winter Rye Seedlings</th>
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</thead>
<tbody>
<tr>
<td>Shoot length</td>
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<tr>
<td>Root length</td>
</tr>
<tr>
<td>Fresh weight</td>
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<tr>
<td>Dry weight</td>
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1 This research was supported by a National Defense Education Act Title IV Fellowship awarded to J. W. and National Aeronautics and Space Administration Grant NGL 12-001-042.
grains in the chloroplasts and apparently functional dictyosomes.

This study then, did not reveal any major difference in ultrastructural detail between cells of H₂O- and D₂O-cultured plants.

LITERATURE CITED

CORRECTIONS

Yoder, O. C. and R. P. Scheffer. Effects of *Helminthosporium carbonum* Toxin on Absorption of Solutes by Corn Roots. Page 518, column 1, “Abstract,” line 10, should be corrected to read: . . . No\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+}, phosphate ion (H\textsubscript{2}PO\textsubscript{4}\textsuperscript{-} and HPO\textsubscript{4}\textsuperscript{2-}), SO\textsubscript{4}\textsuperscript{2-} . . .

Waber, J. and W. S. Sakai. The Effect of Growth in 99.8% Deuterium Oxide on the Ultrastructure of Winter Rye. Page 128, column 2, Table I, should be corrected to read:

<table>
<thead>
<tr>
<th></th>
<th>H\textsubscript{2}O</th>
<th>D\textsubscript{2}O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot length</td>
<td>7.7 ± 1.5 mm</td>
<td>5.3 ± 1.7 mm</td>
</tr>
<tr>
<td>Root length</td>
<td>1.5 ± 0.4 cm</td>
<td>3.8 ± 1.9 mm</td>
</tr>
<tr>
<td>Fresh weight</td>
<td>54 ± 12 mgm</td>
<td>59 ± 9.9 mgm</td>
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<tr>
<td>Dry weight</td>
<td>20.3 ± 3.6 mgm</td>
<td>19.5 ± 3.6 mgm</td>
</tr>
</tbody>
</table>

Duggan, Jeffrey and Merrill Gassman. Induction of Porphyrin Synthesis in Etiolated Bean Leaves by Chelators of Iron. Page 214, column 2, first complete paragraph, line 7, should be corrected to read: In contrast to the situation in algae (2), the molar reduction in porphyrin levels by levulinic acid in higher plant leaves is not stoichiometric with the molar equivalent increase in ALA levels (cf. ref. 24).