Further Studies of the Ultrastructure of $D_2O$ Grown Winter Rye

J. WABER and W. S. SAKAI

Departments of Botany and Agronomy and Soil Science, University of Hawaii, Honolulu, Hawaii, U.S.A.

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Summary

An electron microscopic study of $H_2O$- and $D_2O$-cultured winter rye ($Secale$ cereale L. cv. Winter) seedlings demonstrated that when compared to cells of $H_2O$-cultured plants, cells of $D_2O$-cultured plants contained many ribosomes (both in the cytoplasm and in the plastids), little smooth endoplasmic reticulum and dictyosomes with compressed cisternae and fewer cisternae per dictyosome. Chloroplasts from cells of $D_2O$-cultured plants contained elongate grana with few lamellae per stack and electron dense partitions. Little stroma lamellae was present. However, numerous ribosomes were present in the stroma. Growth in $D_2O$ did not appear to affect microtubule morphology or occurrence.

1. Introduction

The biological effects of deuterium oxide ($D_2O$) have been the subject of many investigations since UREY, BRICKWEEDE, and MURPHY (1932) first discovered the compound. 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 $D_2O$ enriched solution (THOMSON 1963). The general conclusion reached through these experiments was that high concentrations of $D_2O$ are generally toxic to biological systems, however, the mechanisms of this toxicity remained unclear.

Following the successful culturing of several species of green algae in essentially 100% $D_2O$ by CHORNEY et al. (1960), the question of $D_2O$ toxicity and its mechanism was extensively studied by KATZ et al. (1958, 1960) and many other investigators (CHORNEY et al. 1960, FLAUMENHAFT et al. 1965, FLAUMENHAFT, CONRAD, and KATZ 1960 a and b). This work suggested that the effects of $D_2O$ on biological systems could be explained by the kinetic isotope theory. That is, the kinetic imbalance created by the depression of reaction rates for deuterium substituted compounds could account for the effects produced in the test organism by $D_2O$ for $H_2O$ substitution. However, this proposed mechanism cannot account for the
high toxicity of D₂O to higher life forms. For although a wide variety of microorganisms have been successfully cultured in completely deuterated environments, higher plants have proven to be very refractory to culture in D₂O at concentrations greater than approximately 70% (Flaumenhaft 1965). In 1964 however, Siegel and his co-workers (1964) reported that seeds of the grass winter rye were able to germinate and grow in 99.5% D₂O. The observed growth was not vigorous or sustained, and while winter rye did seem to be more resistant to the toxic effects of culture in D₂O than the other organisms tested it certainly was not completely resistant. Siegel and Galston (1966) later used winter rye as a source of deuterium labelled peroxidase. No subsequent investigations with this particular organism have been reported.

This study, an expansion of an earlier report (Waber and Sakai 1974), was an attempt to determine if there was any observable ultrastructural effects of growth in D₂O that could suggest an explanation for the high toxicity of D₂O to higher plants and/or give some indication as to why winter rye is at least partially resistant to this toxicity.

2. Materials and Methods

Winter rye (Secale cereale L., cv. Winter) seeds were surface sterilized and planted in petri dishes on sterile filter paper moistened with either H₂O or D₂O. The dishes were then covered and sealed with tape to prevent any isotopic exchange. The plants were harvested at a time of morphological similarity approximately two days for the H₂O control and nine days for the D₂O grown seedlings.

For electron microscopic study, tissue of the first leaf and coleoptile was diced into 1 mm pieces and placed in formaldehyde-glutaraldehyde fixative (Karnovsky 1965) for 4 hours at 23 °C. Samples were then washed in 0.05 M phosphate buffer (pH 7.2), and post fixed for two hours in one percent osmium tetroxide at about 4 °C. After washing in phosphate buffer, samples were 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 in aqueous two percent uranyl acetate or twenty percent uranyl acetate in fifty percent ethanol followed by lead citrate stain (Reynolds 1963). The sections were viewed and photographed with a Hitachi HS-8-1 Electron Microscope (operated at 50 kV) of the St. John Plant Science Laboratory Electron Microscope Facility.

For light microscopy 0.5 micron thick plastic sections were stained with 0.05% aqueous toluidine blue-0 and then viewed and photographed with a Zeiss RA microscope.

3. Observations

Winter rye seedlings grown in either H₂O or D₂O for approximately 2 or 9 days respectively, appear to be at the same developmental stage. These plants have a well developed root and a mature coleoptile which encloses the first developing leaf. The two types of seedling also have a similar anatomy (Figs. 1 A and B). Cross-sections of the first leaf show the occurrence of cell types and the arrangement of epidermis, mesophyll and vascular