DEUTERIUM NON-EVAPORATIVE FRACTIONATION OF WATER INTO THE EMBRYONIC AND NON-EMBRYONIC CARROT (DAUCUS CAROTA) CELLS

P. Berdea¹, N. Palibroda¹, Stela Cuna¹, and C. Deliu²

¹ National Institute of Research - Development for Isotopic and Molecular Technology, Cluj-Napoca, Romania
² Biological Research Institute, 48 Bilaşcu st., RO - 3400 Cluj-Napoca, Romania

We report for the first time a water deuterium isotopic fractionation between extracellular water and cellular water for in vitro grown carrot (Daucus carota) cells. The deuterium was found to be higher within the cells by 10 ‰ for non-embryonic cells and 13 ‰ for the embryonic cells. Such a non-evaporative fractionation of water in the cells is an additional step to the already well known evaporative fractionation in the plants. Because the main source of hydrogen for organic matter is the internal water of the cells, deuterium fractionation of water during its passing throws the cellular membrane has wide relevance for biochemical, physiological processes, and environmental studies.

1. INTRODUCTION
The investigation of deuterium content in extant or fossil plant organic material is of interest for various problems of the plant metabolism, in paleoclimatology and environmental studies [1].

The natural hydrogen presents two stable isotopes, the hydrogen and the deuterium.

The natural content in deuterium is expressed as:

\[ R = \frac{D}{H} \]  

where: \( D \) - number of deuterium atoms; \( H \) - number of hydrogen atoms

The deuterium content in different materials are generally expressed as “\( \delta \) value” in parts per thousand. The \( \delta \) value is defined as :

\[ \delta_D = (\frac{R}{R_s} - 1) \times 1000 \]  

where: \( R \) - the deuterium / hydrogen ratio of the sample; \( R_s \) - the isotope ratio of the international standard V-SMOW (Vienna Standard Mean Ocean Water) [2].

Many chemical and physical processes have a significant isotopic fractionation, which generally refers to an enrichment or depletion of the heavy (deuterium) isotope. An isotope effect, referred to as the fractionation factor for the equilibrium reaction \( A \leftrightarrow B \) defined as \( \alpha_e = \frac{R_A}{R_B} \), or in \( \delta \) notation, \( \alpha_e = \frac{(1000+\delta_A)}{(1000+\delta_B)} \), were \( A \) refers to reactant and \( B \) to the product. In the kinetic reactions, the kinetic fractionation factor, \( \alpha_k \), is an instantaneous measure of isotopic fractionation.

The isotopic fractionation is also defined as:

\[ \Delta = \delta_A - \delta_B \]
where: \( \delta_A \) - deuterium content of the reactant; \( \delta_B \) - deuterium content of the product.

The deuterium fractionation between soil water and plant cellulose is of interest for biochemical, paleoclimatological, physiological and environmental studies. For example the D/H ratio of the cellulose nitrate from nowadays or fossil plants gives information about the climate when the cellulose was synthesised. As for biology the ratio D/H is of interest because it provides information about plant physiology, plants biochemical processes and the environment where the plants lives in. The major information about D/H isotopic fractionation in plants has been derived from the naturally growing plants, manly higher plants in which the causes of the isotopic composition are difficult to interpret because of complexity of internal and external factors. This water inside the cells is the source of hydrogen for plant organic matter. The water is believed to present no fractionation at it passing through the cell membrane [3].

Our results showed non-evaporative fractionation between intracellular water and extracellular water, in embryonic and non-embryonic carrot (Daucus carota) cells.

2. MATERIALS AND METHODS

The cells have been exposed to a water solution with an uniform isotopic content. The carrot (Daucus carota) cells were grown in vitro, in a Murashige and Skoog mineral-salts medium [4] with 30 g/l sucrose and 1 mg/l 2,4-dichlorfenoxiacetic acid. The pH of solution was 5,7 before autoclaving at 120 °C. The cells were grown in sealed glass bottles.
continuously agitated at 98 rot./min., with a diurnal cycle of 16 hours in the light, and 8 hours in the dark at 25°C controlled temperature. The initial cells density was 1.8 (g dry weight)/(l culture medium) for non-embryonic and 1(g dry weight)/(l culture medium). The non-embryonic culture densities expressed as (dry weight)/(culture medium volume) were: 13.4 g/l for 7 days old cells, 17.3 g/l for 14 days old cells and 18.6 g/l for 21 days old cells. The culture densities of embryonic cells were: 5.6 g/l for 7 days old, 9.8 g/l for 14 days old and 11.2 g/l for 21 days old cells. Seven days after inoculation of cells in bottles with 30 ml culture solution, the cells culture from 3 bottles containing 1/3 of entire solution was filtered and cells were captured on filter paper. The cells water was vacuum extracted. A portion of distilled water of culture medium was kept for D/H isotopic analysis. The water from filtered aqueous solution and the cell water were analysed by mass spectrometry [5]. The procedure was repeated for 14 days and 21 days old cell cultures. The whole experiment was repeated three times. Experimental error was: ± 30/00 for δD = [(D/H)x/(D/H)s - 1] 1000, where X refer to the sample and S refer to the SMOW (Standard Mean Ocean Water). The measured δD values were in a normalised VSMOW-SLAP scale [6].

3. RESULTS AND DISCUSSIONS

The purpose of our work was to determine the water deuterium isotope fractionation when passing through the cell membrane. The
deuterium content of starting water and the deuterium content of final culture water, $\delta D_{EW}$ is the same, in the limits of experimental errors.

The experiment shows deuterium non-evaporative fractionation of non-embryonic and embryonic carrot cells for 7 days, 14 days old cells and 21 days old cells (Table 1).

The deuterium fractionation of water is:

$$\delta D_W = \delta D_{IW} - \delta D_{EW}$$

(4)

where, $\delta D_{IW}$ is the deuterium content of internal water of the cells and $\delta D_{EW}$ is the deuterium content of external water (water of culture medium). The deuterium fractionation factor of water in the cells is:

$$\delta_{CW} = \frac{1000 + \delta D_{IW}}{1000 + \delta D_{EW}}$$

(5)

Table 1

The average deuterium fractionation of water was $10^0/00$ for non-embryonic and $13^0/00$ for embryonic carrot cells for up to 14 days old cells. The fractionation diminished for 21 days old non-embryonic and embryonic carrot cells. This is due to the fact that the optimal time from carrot cell culture in vitro is 14 days. After 21 days many cells were dead and their internal waters and extracellular waters were mixed. Consequently, the deuterium content of the internal water for the 21 days old cells diminished.

The deuterium source for plants is the environmental water [7]. Water is taken in the roots and move upward in the xylem to the leaves. Transpiration from plant leaves leads to fractionation of xylem water. As a result, leaf water is considerable enriched in deuterium. The deuterium leaf water is used in the biochemical reaction during photosynthesis for organic matter production. The major structural carbohydrate in terrestrial and many
aquatic plants is the cellulose. Paleoclimatologist have measured deuterium content, δD of cellulose nitrate which contain only non-exchangeable carbon-bound hydrogen from modern and fossil plants because this deuterium content record information about the climate when the cellulose was synthesised. The relationship between deuterium composition of cellulose nitrate δD_{CN} and deuterium composition of meteoric water δD_{MW} is:

$$\frac{\delta D_{CN} + 1000}{\delta D_{MW} + 1000} = \alpha_n \alpha_c \alpha_k \alpha_n (\alpha_e \alpha_k - 1) h$$  \hspace{1cm} (6)

where: $\alpha_n$ is the biochemical fractionation factor between cellulose and leaf water, $\alpha_c$ and $\alpha_k$ are the fractionation factor at equilibrium and the kinetic fractionation factor between liquid and vapour, and $h$ is the relative humidity. The equation (6) is valid only if the source of cellulose is the leaf. Leaf water is assumed to be an isotopically uniform pool. A few workers inferred the possibility of isotopic inhomogeneity within leaves. Difference between the δD values of waters from white pine (Pinus strobus) leaves by vacuum distillation and by pressing was reported [8]. A more detailed discussion, on this can be found [9, 10].

In the carrot cells growing in water solution, as aquatic plants the equation (6) is reduced to:

$$\alpha = \alpha_n$$  \hspace{1cm} (7)

because the humidity factor $h=1$, and without water evapo-transpiration (fractionation factors $\alpha_e=1, \alpha_k=1$).

The overall deuterium fractionation factor, $\alpha_n$ in the carrot cells is:

$$\alpha_n = \alpha_b \alpha_{cw}$$  \hspace{1cm} (8)
where: $\alpha_b$ - biological fractionation factor; $\alpha_{cw}$ - the deuterium fractionation factor of cell water.

The non-evaporative fractionation of water in aquatic organisms plants has also been reported [11]. The deuterium content of the thallus water is by about 5-10 ‰ lower than the ocean water [12]. The non-evaporative fractionation between intracellular water and extracellular water in embryonic and non-embryonic carrot (Daucus carota) cells represents a new step in the overall fractionation of deuterium in the cells.

The possible explanation for the enrichment or depletion of deuterium in cell water is the cell cycle regulation role of the naturally occurring deuterium [13]. In experiments with deuterium enriched water the major effect of deuterium was the inhibition of the cell division, and depletion of deuterium in tissue water and organic mater [14]. The deuterium-depleted water (30 ppm) also decreased the growth rate of the L929 fibroblast cell line [13]. The authors suggest that the naturally occurring deuterium is essential for cell cycle regulation.

The existence of an isotopic fractionation through the cell membrane implies that the relationship between the deuterium content of cellulose nitrate and meteoric water, according to equation (6), should be revised.

This work represents the first attempt to investigate deuterium fractionation in water when passing to cells membranes. We proved the existence of such an effect for carrot cells in vitro. As a result the inner water deuterium content $\delta D_{IW}$ is higher than the external water deuterium content $\delta D_{EW}$. This finding is of interest for the understanding of some
biochemical processes as well as for the balance and dynamics of the hydrogen isotopes in the environment because this water inside the cells is the major source of hydrogen and oxygen for the synthesis of the plant organic matter. The non-evaporative fractionation is a new step in the overall fractionation of water in the plants.

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REFERENCES


Table 1

Deuterium non-evaporative fractionation of water in the non-embryonic and embryonic carrot (*Daucus carota*) cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\delta$D$_{IW}^a$ (%)</th>
<th>$\delta$D$_{EW}^b$ (%)</th>
<th>$\delta$D$_W^c$ (%)</th>
<th>$\delta$C$_d$</th>
</tr>
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<tbody>
<tr>
<td>non-embryonic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days old</td>
<td>-91.2±3.1</td>
<td>-101.5±2.7</td>
<td>10.27</td>
<td>1.0114</td>
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<tr>
<td>14 days old</td>
<td>-90.9±2.8</td>
<td>-101.5±2.7</td>
<td>10.59</td>
<td>1.0118</td>
</tr>
<tr>
<td>21 days</td>
<td>-92.5±2.9</td>
<td>-101.5±2.7</td>
<td>8.99</td>
<td>1.010</td>
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</tbody>
</table>

65
<table>
<thead>
<tr>
<th>Age</th>
<th>δD&lt;sub&gt;IW&lt;/sub&gt; (‰)</th>
<th>δD&lt;sub&gt;EW&lt;/sub&gt; (‰)</th>
<th>δD&lt;sub&gt;W&lt;/sub&gt;</th>
<th>δC</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 days</td>
<td>-88.3±2.7</td>
<td>-101.5±2.7</td>
<td>13.16</td>
<td>1.0146</td>
</tr>
<tr>
<td>14 days</td>
<td>-88.3±2.9</td>
<td>-101.5±2.7</td>
<td>13.22</td>
<td>1.0147</td>
</tr>
<tr>
<td>21 days</td>
<td>-91.6±3.1</td>
<td>-101.5±2.7</td>
<td>9.95</td>
<td>1.011</td>
</tr>
</tbody>
</table>

\( \delta D_{IW} \) - the deuterium content of cell water (inner water)

\( \delta D_{EW} \) - the deuterium content of the external water

\( \delta D_{W} \) - the deuterium water fractionation in the carrot cells

\( \delta C \) - the deuterium water fractionation factor in the carrot cells.