Note

Water and Potassium Ion Absorption by Deuterium Oxide Resistant Winter Rye Seedlings

Sadami SHIBABE and Karin YODA
Radioisotope and Nuclear Engineering School, Japan Atomic Energy Research Institute
28-49, Hon-komagome 2-chome, Bunkyo-ku, Tokyo 113, Japan
Received February 12, 1985

The concentrations of deuterium oxide (D₂O) in the root tissue water of winter rye seedlings equilibrated with the external D₂O concentration within 30 min and in the shoot tissue water after 5 - 6 h. The equilibrated value in the root water was 87% and in the shoot water, 55% of the external concentration. The K⁺ absorption rate of the seedlings decreased from a value of 39 to 18 μmol g⁻¹ h⁻¹ when the D₂O concentration was changed through a range from 0% to 97%. D₂O suppressed the absorption of water and K⁺ by the seedlings. The higher the D₂O concentration the greater the suppression, but it was less than with similarly treated rice plants. However, the process of D₂O absorption by the seedlings was similar to that of rice seedlings (Envir. exp. Bot., 24, 369).

Key Words: winter rye, water absorption, potassium ion absorption, deuterium oxide, isotope effect

1. Introduction

Among the higher plants winter rye is known to be resistant to deuterium oxide (D₂O): Winter rye is able to germinate and grow in 99% D₂O without the prior adaptation usually found to be required. Some biochemical processes of winter rye are known to be affected by D₂O, but the physiological features have not yet been clarified as they have been in the case of rice.

Rice, which belongs to the same family Gramineae as rye, is affected greatly by D₂O on the absorption processes of water and ions, and on the germination process of seeds.

We aimed to further illustrate 'the resistance' of rye with finding how the absorption of water and ions is affected by D₂O.

2. Materials and Methods

Seedlings of winter rye, Secale cereale cv. Petkusa, were raised with tap water for 7 to 8 days at 25°C and r.h. of 60 - 70% under a 14 h photoperiod with 12 000 lx. The seedlings were rinsed in deionized water and then maintained in defined concentrations of D₂O (99.83%, Showa Denko) containing 1 × 10⁻⁴ mol l⁻¹ CaSO₄ and for experiments with tritium, 4 kBq ml⁻¹ (0.1 μCi ml⁻¹) tritium oxide (HTO) for absorption periods.

At hourly intervals bundles of the seedlings were withdrawn from culture solutions for 'water' absorption experiments. The analysis of D₂O and/or HTO content was carried out by the method described previously.

The distribution of D₂O and/or HTO in plants equilibrated with culture solutions, was measured in shoot sections of 2, 2, 3, 4 and 5 cm-long along the direction from shootbase toward the top. The sections were separately stored in plugged test tubes at −25°C for isotope analysis.
Potassium ion absorption experiments were carried out by the method described previously\(^8\).

About 50 seeds of winter rye were germinated on the absorbent cotton sheets saturated with D\(_2\)O solutions on Petri dishes in darkness at 25°C. The germination of the seeds was counted once or twice a day.

The figures and table represent the average determination of two duplicate experiments.

3. Results and Discussion

The time course of D\(_2\)O absorption by winter rye seedlings is shown in Fig. 1. The D\(_2\)O concentration of root tissue water attained equilibrium with that of culture solutions within 30 min. The equilibrium value of the root tissue water, however, was different from the external D\(_2\)O concentrations, i.e. about 87\% of the external concentrations from 0\% to 97\% D\(_2\)O. The amount of D\(_2\)O translocated from roots to shoots increased sigmoidally with time and approached an asymptote after 5–6 h. However, the saturation levels in shoot tissue water after 5 h was 55\% of the external D\(_2\)O concentra-

Fig. 1 Time course of D\(_2\)O absorption by rye seedlings with the D\(_2\)O solutions of 0.0\% ○, 58.6\% □ and 97.3\% △. At 0.0\% D\(_2\)O 1.5 kBq ml\(^{-1}\) (4 nCi ml\(^{-1}\)) HTO was added to the culture solution. The values of D\(_2\)O and HTO concentration in the tissue water are expressed in a ratio, based on the D\(_2\)O- and HTO-concentration of culture solutions as unity, respectively.

Fig. 2 Distribution patterns of HTO in co-existence with D\(_2\)O in shoot tissue water of rye seedlings. The seedlings were immersed 14 h in culture solutions of 0.0\% ○, 20.5\% ▽, 38.4 \% ◊ and 96.0% D\(_2\)O △, containing 4 kBq ml\(^{-1}\) (0.1 µCi ml\(^{-1}\)) HTO.

It is known that a trace of HTO moves together with the flow of various concentrations of D\(_2\)O in rice plants\(^6\), so the HTO movement in rye seedlings maintained with 0\% D\(_2\)O is considered to represent the water flow of natural D\(_2\)O level.

Figure 2 shows the distribution patterns of D\(_2\)O in shoot tissue water in relation to distance from the shootbase. When the seedlings were equilibrated with culture solutions overnight, the D\(_2\)O concentration of tissue water was high at the shootbase and low at their tops. At the shootbase the concentration was 83\% of the various culture solutions and almost coincided with the 87\% concentration of the root tissue water (Fig. 1). The D\(_2\)O concentration remained practically unchanged from the base to the top of the protective sheath (coleoptyle apex). At the upper parts of the sheath the concentration decreased sharply toward the leaf apex and reached a value less than 20\% of
Table 1 Decrease in water content and hydrogen isotope concentrations of shoot tissue water of winter rye seedlings exposed to air for 3 hours

<table>
<thead>
<tr>
<th></th>
<th>Initial (A)</th>
<th>Final (B)</th>
<th>B/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>D$_2$O (Water content)*</td>
<td>30.7 atom% D (87.6%)</td>
<td>27.6 atom% D (84.5%)</td>
<td>0.899</td>
</tr>
<tr>
<td>D$_2$O (Water content)*</td>
<td>45.9 atom% D (88.0%)</td>
<td>36.7 atom% D (85.1%)</td>
<td>0.800</td>
</tr>
<tr>
<td>HTO (Water content)*</td>
<td>4.28 $\times$ 10$^3$ dpm/ml (86.5%)</td>
<td>3.60 $\times$ 10$^3$ dpm/ml (83.0%)</td>
<td>0.841</td>
</tr>
</tbody>
</table>

* On fresh weight basis

Fig. 3 Time course of the absorption (a) and the translocation (b) of potassium ions by rye seedlings in culture solutions of 0.0% O, 20.4% ●, 38.0% △, 57.7% ▲ and 97.4% D$_2$O ||, of pH 5.8 - 6.2 at 25°C. All the culture solutions contained 2$\times$10$^{-4}$ mol l$^{-1}$ KCl with 7.8 hBq ml$^{-1}$ (2.1$\times$10$^{-8}$ Ci ml$^{-1}$) of $^{42}$K and 1$\times$10$^{-4}$ mol l$^{-1}$ CaSO$_4$. The seedlings were pretreated with D$_2$O solutions excluding K$^+$ for 14 h before beginning the absorption period.

that of the external solutions. The distribution pattern did not change over the D$_2$O concentration of culture solutions ranging from 0% to 96%.

Table 1 shows the decrease in D$_2$O and HTO concentrations of tissue water in the shoots excised from roots and left in air for 3 h. As was expected, the water content in the shoots decreased by about 4%, but D$_2$O and HTO concentrations decreased more greatly, by about 15%. The concentration of a trace of HTO in the shoot tissue water decreased in a similar way to a large amount of D$_2$O.

These facts described above lead to the same conclusion as was drawn from the rice seedling experiments—i.e. that about 10% of the tissue water in whole seedlings is unexchangeable, and the water translocated to leaves is diluted rapidly with aerial H$_2$O.

Potassium ion absorption by winter rye seedlings depended distinctly on D$_2$O concentration as shown in Fig. 3. The amount of K$^+$ absorbed by the roots increased linearly with time, over the first 30 min. The rate of K$^+$ absorption was estimated from the slope of the curves in Fig. 3(a). The absorption rate decreased from a value of 39.3 to 34.4, 29.3, 24.5 and 17.6 $\mu$mol g$^{-1}$ h$^{-1}$ for D$_2$O concentrations of 0, 20.4, 38.0, 57.7 and 97.4%, respectively.

This suppression is well demonstrated in Fig.
Fig. 4 D$_2$O suppression curves of potassium ion absorption and germination of winter rye. Relative K$^+$ absorption rates were obtained from the slopes of the curves in Fig. 3(a). Relative germination rates were expressed as "the time required for 50% germination" obtained from germination curves. Broken line shows the result on K$^+$ absorption by rice seedlings$^8$.

4. The D$_2$O suppression curve for K$^+$ absorption by the roots, was linear on a semilog scale and had a gentle slope compared with that of rice seedlings$^9$. The degree of suppression was not as great as that of rice seedlings. This means that in regard to ion absorption rye seedlings are more resistant to D$_2$O than are rice seedlings. The linear relation between the degree of suppression of the ion absorption and the D$_2$O concentration suggests that there is no threshold of D$_2$O concentration at which suppression occurs.

The amount of K$^+$ translocated from the roots to the shoots increased exponentially with absorption time (Fig. 3(b)). Potassium ion translocation was depressed according to the level of D$_2$O. With less than 40% D$_2$O, the translocation was merely delayed, but with higher than 60% D$_2$O almost no translocation occurred.

It was reconfirmed the finding by Siegel, et al.$^9$ that rye seeds were capable of germinating in 99% D$_2$O. We observed, however, that their germination rate was delayed with higher concentrations of D$_2$O, e.g., based on the 50%-germination level obtained from the germination curves of the seeds, it changed from 15 h at 0% D$_2$O to 52 h at 99% D$_2$O. The retardation is shown as a sigmoidal curve in Fig. 4. It means that the degree of the retardation can be expressed as an exponential function of D$_2$O concentration similarly to that of the ion absorption described above.

It has been reported that the rye seeds germinated in D$_2$O are able to synthesize de novo isoperoxidases similarly to the seeds germinated in H$_2$O$^9$. The protein synthesis of the seedlings of winter rye grown in 99% D$_2$O is partially blocked$^9$. Hence the 'D$_2$O resistance' of winter rye may be explained by detailed investigations on the biochemical processes of the plants.

It was concluded that (1) the physiological processes of rye plants, e.g., the water and ion absorption by the seedlings, are suppressed by D$_2$O, and (2) the degree of suppression depends on the D$_2$O concentration, as was reported for rice plants$^{5-9}$, however, (3) the process of D$_2$O absorption by the seedlings is substantially similar to that by rice seedlings$^9$.

We thank Dr. T. Miura of the Hokkaido Prefectural Breeder's Stock Farm for generously providing us with the seeds of a cv. Petkusa of winter rye.

References