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DIFFUSIBLE AND EXCHANGEABLE RUBIDIUM IONS IN PEA ROOTS

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INTRODUCTION

Since the concept of apparent free space or outer space plays every day a greater part in the research on ion uptake (BRIGGS and ROBERTSON, 1958), many investigators are inclined to assume that the ions present in the apparent free space are carried passively by the transpiration stream as a kind of mass transport (a.o. HYLMÖ 1953; EPSTEIN 1956; KRAMER 1957; KYLIN and HYLMÖ 1957).

Whereas apparent free space is by definition freely accessible for all substances dissolved in the outer solution, this space would also have a free communication with the xylem vessels. This would mean that there does not exist an ion barrier between medium and xylem vessels. The ions would, therefore, be able to move freely by means of diffusion across the root tissue.

There are, however, many data which present difficulties to this concept. It seems, in the first place, to increase the difficulties of explaining the bleeding phenomenon, an objection against their theory that has already been mentioned by the authors of the view cited above (EPSTEIN 1956; KRAMER 1957). It is difficult to see how ions could be accumulated in the xylem vessels if we were to accept this hypothesis, but even when it takes place in the way suggested by HYLMÖ (1953) viz. by accumulation in the vacuoles of differentiating xylem elements, which cells would release the ions in the xylem sap, it remains obscure how a higher concentration of ions in the xylem vessels could be maintained if a free passage of ions across the root tissue occurs. For, in that case we have to assume that an ion concentration, however it may be effected, would be leveled down very rapidly.

A second objection against the theory cited above has been given by means of experiments in which it was shown, that ions formerly accumulated in the cells, can be transported from cell to cell without leaching out to the medium. This has been shown to occur in Vallisneria leaf tissue (ARISZ 1954) and for the transport of ions from the root to the shoot (HELDER, 1958).

These observations would tend to make one cautious in regard to the rather simple and perhaps obvious conclusions to be made from

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the free space experiments. The experiments described in this paper will show how exchangeable rubidium ions present in the roots can be easily translocated to the shoots without important leaching out to the distilled water in which the root systems were placed.

Material and methods

After germination in moist sand pea seedlings were mounted on wooden disks (sets of 18 seedlings on each disk were used) and cultivated in a glass-house on a half-strength Hoagland solution for 18–25 days. They were then used for the experiments.

During the uptake period, the root systems of intact plants or excised root systems were placed on a labelled rubidium chloride solution with constant aeration. After that, part of the sets were rinsed during 10 or 20 seconds in distilled water by moving them 2 or 6 times up and down, blotting them then with filterpaper. The amount of labelled rubidium present in the root systems treated this way is taken as being the ions in the apparent free space (FSI) + exchangeable ions (EI) + accumulated ions (AI).

Another part of the root systems was rinsed with distilled water during 30 to 240 minutes (time of rinsing mentioned for each experiment) and the rubidium ions present in the tissue after this treatment are taken as being exchangeable ions + accumulated ions. The accumulated fraction is defined as the amount of rubidium present in the root after washing the root systems in an unlabelled solution with the same concentration as used during the uptake period.

By subtracting the different amounts from each other, the values for apparent free space ions, exchangeable fraction and accumulated fraction were determined.

Experiments

1. *The amount of ions in the different fractions as dependent on the duration of uptake.*

Excised root systems were transferred to a 10 mM Rb*Cl solution and after various absorption periods taken out for analysis. Each time the series consisted of three sets. One was washed in distilled water during 20 seconds by gently moving the root system up and down 5 times and thereafter blotting them with filterpaper. Another set was rinsed in distilled water during 60 minutes and the third ones in 10 mM RbCl during the same time. Fig. 1 shows the amounts of Rb* ions present in the roots after the different treatments as dependent on the duration of the uptake period.

The accumulation of Rb* ions in the root tissue continues at a constant rate throughout the whole experiment. Apparent free space and exchange space, however, are fully equilibrated with the outer solution in time periods as short as 30–60 minutes. This is shown in Fig. 2. The amount of ions in the AFS and the amount of EI are more or less the same in this experiment.

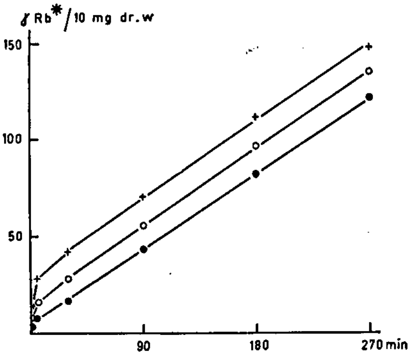

Fig. 1.

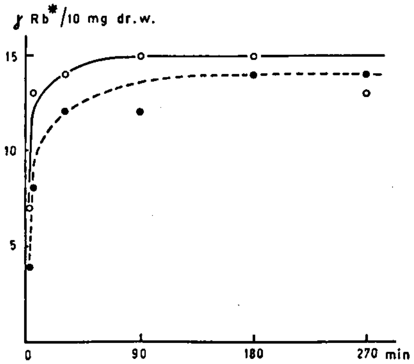

Fig. 2.

Fig. 1. Time course of the Rb* uptake by excised pea roots. Nutrient solution 10 mM Rb*Cl. Aeration throughout.

Fig. 2. Time course of the penetration of the root (Fig. 1) by FSI (circles) and EI.

2. *Further analysis of the exchangeable fraction.*

In previous experiments the amount of exchangeable rubidium was rather variable. Moreover there was a striking difference in the bleeding activity. Sometimes the root systems showed a distinct bleeding, in other cases the bleeding was very small. It was to be suspected that there would be a relation between the variability in bleeding activity and the amount of exchangeable ions in such a way, that root systems showing rapid bleeding in distilled water would contain a smaller amount of exchangeable Rb* afterwards. The root systems which were rinsed in distilled water with the cut surface below the surface of the liquid were now washed, partly with the cut surface below and partly with this surface above the solutions. Fig. 3 shows the results of such experiments. After the uptake period, part of the root systems were firmly blotted with filterpaper (FSI + EI + AI vide p. 69), another part was rinsed with distilled water during 60 minutes, i.e. group 1 with the cut surface above and group 2 with the cut surface below the water surface. The third portion was washed during 60 minutes with the unlabelled solution in the same manner, i.e. with the cut surface above or below the surface.

It appears (Fig. 3), that part of the ions which are measured as exchangeable ions in group 1 are measured as AFS-ions in group 2. This means that part of the exchangeable ions leaves the root through the cut surface if this surface is rinsed in water. This fraction may be present in the xylem vessels at the end of the uptake period and/or it represents the results of the bleeding of the root system.

In the following experiment the bleeding sap was captured and analysed separately, whereas the rootstump above the rinsing solution (about 1. cm) was analysed separately from the rest of the root tissue. The results of two experiments of this kind were plotted in Fig. 4. The differences arising from rinsing with and without uranylnitrate 10^{-3} M during a rinsing period of 45 minutes were compared.

It is clear that without inhibitor more Rb* is transported to the rootstump and more is excreted in the bleeding sap than in the presence of the inhibitor. Comparing these two treatments it is clear that the transported labelled rubidium ions are totally removed from the exchange spots and partly from the accumulated fraction (negative exchangeable fraction) without inhibitor.

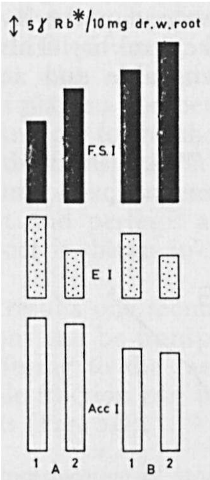

Fig. 3.

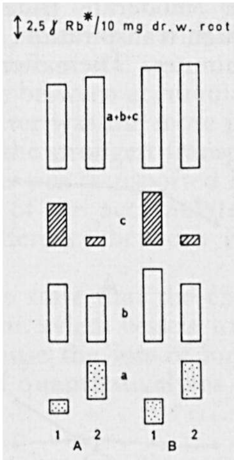

Fig. 4.

Fig. 3. Influence of the way of rinsing on the amount of Rb* ions in the different fractions viz. accumulated ions (Acc.I.), exchangeable ions (EI) and ions in the free space (FSI). 1. rinsed with the cut surface above the liquid. 2. rinsed with the cut surface immersed. A and B replicate experiments.

Fig. 4. The bleeding as a cause of the decrease in amount of exchangeable ions during a stay on distilled water. After an uptake period on 5 mM Rb*Cl during 3 hours, the roots have been rinsed 45 minutes in distilled water (1) or in 10^{-3} M uranylnitrate (2). In both cases with the cut surface above the liquid. *a.* exchangeable Rb* ions in the root tissue after the rinsing period. *b.* Rb* ions in the root stump i.e. that part of the root above the liquid surface. *c.* Rb* ions in the exudate.

Summarising the results of the experiments described in this section it is evident, that the exchangeable ions can be quantitatively transported with the bleeding stream, and that an active process takes the ions from the exchange sites into the xylem vessels (ARISZ 1945, 1956, 1958).

3. *The translocation of exchangeable rubidium ions to the shoot.*

In the preceding paragraph we have seen, that the exchangeable ions can be translocated to the xylem vessels which causes an exudation at the cut surface of decapitated plants. It will be of some interest to know how this transport depends on the transpiration rate in intact plants. In the following experiment (results plotted in Fig. 5) intact pea plants absorbed Rb*Cl during 60 minutes. At the end of this period the shoots of part of these sets were excised and the roots were rinsed with distilled water or with unlabelled rubidium chloride. In this manner the amount of diffusible, exchangeable and accumulated ions at the end of this period can be known, the state of affairs being shown in Fig. 5*A*. Of the other sets the root systems were transferred to distilled water and the shoots were covered by a glass-cover (low transpiration, water uptake 2 ml/hr), or placed at free transpiration (moderate transpiration, water uptake 4 ml/hr) or ventilated (high transpiration, water uptake 7 ml/hr). This treatment lasted 60 minutes. Thereafter the exchangeable and accumulated

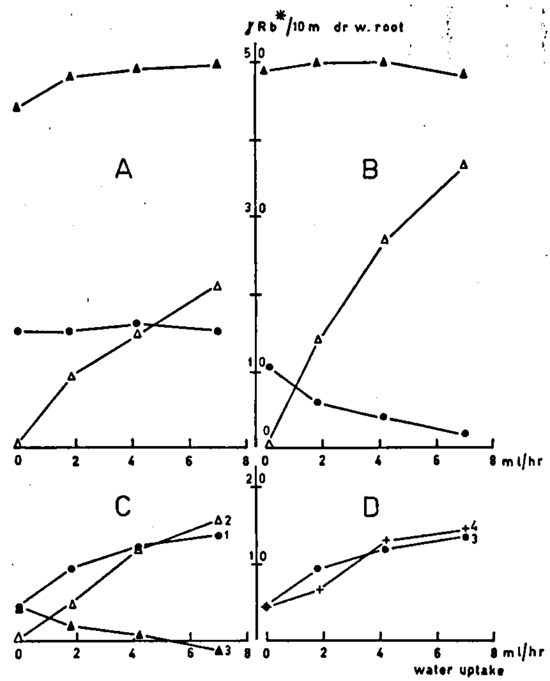

Fig. 5. Uptake and subsequent redistribution of Rb* ions in pea plants.
A. The amount of Rb* ions in the shoots (△ — △) and in the roots, the latter separated in exchangeable ions (● — ●) and accumulated ions (▲ — ▲) as measured directly after an uptake period of 1 hour on 10 mM Rb*Cl at various transpiration rates (0 ml/hr = excised root systems, 1.84 ml/hr glasscovered intact plants, 4.16 ml/hr freely transpiring plants and 7.00 ml/hr).
B. The distribution of the Rb* ions over the three fractions of Fig. 5*A* after a 1 hour uptake period on 10 mM Rb*Cl followed by a subsequent stay of 1 hour on distilled water. Transpiration treatment of the plants during both periods as indicated on Fig. 5*A*.
C. Changes in the amount of Rb* ions in the three fraction (differences between Fig. 5*A* and Fig. 5*B*) during the stay on distilled water as related to the transpiration rate. (1) decrease in the amount of exchangeable Rb* ions in the roots. (2) increase in the amount of Rb* ions in the shoot. (3) increase or decrease of the amount of accumulated Rb* ions in the roots.
D. Showing the accordance between the decrease of the exchangeable fraction (3) and the increase in accumulated + transported fraction (4).

fraction was determined in the same way as described above. The state of affairs at the end of the redistribution period is plotted in Fig. 5*B*.

It became apparent that the treatment following the uptake period resulted in a decrease of the amount of exchangeable labelled rubidium ions in the roots and that this decrease was greater with high transpiration rates than with low transpiration, the moderate transpiration lot giving values in between. In the case of the decapitated root systems on distilled water, the decrease of the exchangeable fraction corresponded with an equivalent increase of the accumulated ions. In the intact plants a competition occurs between accumulation in the root and transport to the shoot. The latter was the more pronounced the greater the transpiration rate, with the strongest transpiration the whole amount of exchangeable Rb* ions was transported in one hour to the shoot and perhaps also a little of the accumulated fraction. This transport is shown in Fig. 5*C* (difference between Fig. 5*A* and Fig. 5*B*).

By these results one seems justified to state that the exchangeable rubidium ions can be transported via the xylem vessels to the shoots without diffusing to the medium, because the loss of ions from the exchangeable fraction can be measured quantitatively as an increase in the shoots (Fig. 5*D*).

4. *The translocation of labelled rubidium ions from the roots to the shoots if both diffusible and exchangeable ions are present.*

The uptake period in this experiment again lasted 60 minutes. After that the root systems of the intact plants were gently blotted with filterpaper (the plants remained in their position on the wooden holders). Three sets were used for the direct estimation of FSI, EI and AI in the way described above. The other sets were placed in the dark with their root systems in humid air, loss of ions to the medium being impossible. After resp. 1, 2 and 4 hours three sets each time were removed for analysis of the three fractions at that moment. At the same time a series of plants remained on the radioactive rubidium chloride solution in order to be able to compare the translocation rates of the series. The results shown in Fig. 6*A* indicate that in this experiment the AFS is considerably greater than in the experiments described above. This was caused by the less effective blotting. The amount of ions in the AFS decreases continuously throughout the experiment and rectilinearly with the time. Besides it is evident that the amount of exchangeable ions did not alter. This means that in this case the transport to the shoot takes place at the cost of the ions in the AFS and not of the exchangeable ions. It remains possible, however, that the exchangeable ions are transported but are replaced from the AFS. On the basis of these experiments it is not possible however to make a choice between these two possibilities.

Comparing the results of the two series, it is evident that the rate of accumulation in the roots is somewhat greater for the plants remaining in the nutrient solution than for those with the roots in humid air. Both rates are smaller than the absorption rates during the first hour, with the shoots in the light.

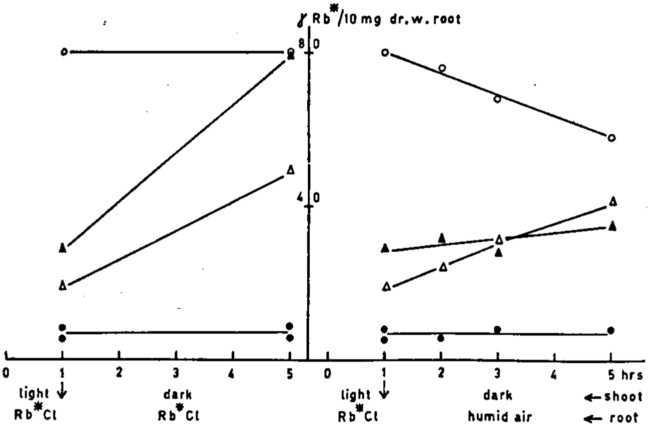

Fig. 6*A*. Amount and distribution of Rb* ions in shoots (△ — △) and roots, the latter separated in accumulated ions (▲ — ▲), exchangeable ions (● — ●), and ions in the free space (○ — ○) after a 1 hour uptake period in the light on a 10 mM Rb*Cl solution and after a subsequent 4 hour period in the dark on the same solution.

Fig. 6*B*. Amount and distribution of the Rb* ions in shoots and roots after an initial uptake period of 1 hour in the light on a 10 mM Rb*Cl solution followed by blotting of the root systems and a subsequent redistribution period in the dark with the root systems in moist air. Signals as in Fig. 6*A*. The ions present in the free space are moving to the shoots and for a part also to the accumulated fraction of the roots. The amount of exchangeable ions remains the same throughout.

Discussion

The data obtained in these experiments are in part in agreement with the results obtained by other authors. After transferring the root systems to a salt solution from a solution lacking these ions the AFS is filled up with ions in a very short time by means of diffusion. This AFS can represent a considerable percentage of the tissue, viz. 20–35 % of the root tissue of barley and wheat (Butler, 1953; Epstein, 1956; Kylin and Hylmö 1957) and a somewhat lesser percentage of bean plants (Hope and Stevens 1952) and pea plants (Hylmö 1953). The percentage found in the present author's experiments with pea plants treated in the way described above were about 7–10 % of the root volume. In accordance with the results of Epstein (1955) the ions present in the free space can be transported to the shoot or accumulated in the root cells.

In addition to the apparent free space another ion fraction is attained at the same time and with the same rate. This fraction may relate to ions adsorbed on conversely charged sites as generally suspected. It is common knowledge that the exchangeable ions can only diffuse from the tissue to the medium if they are replaced at the same time by ions of the same kind or by very similar ions. By such a behaviour this ion fraction is defined and this is used to measure this component.

Most interesting in these experiments the present author considers the fact that during a stay on distilled water following an uptake period the exchangeable rubidium ions are released from the root tissue. These ions are, however, not given off to the outer solution but are quantitatively involved in a one-way transport to the exudation sap or to the shoot. This means that these ions once in the protoplasm go much easier towards the xylem vessels than back towards the medium.

If both diffusable and exchangeable ions are present, the amount of the latter is nearly constant. This means that the diffusible ions either go straight to the xylem vessels or they replace the exchangeable ions if these are directly transported to the xylem vessels. At the moment no preference can be given to one of these two possibilities.

These experiments also give some suggestions on the localisation of the ion fractions concerned. The observation that the amount of ions in the AFS seems to be greater when the root systems are rinsed while submersed in water than when rinsed with the cut surface above the liquid and that the exchangeable fraction in the latter case is greater, is a strong indication that exudation is continued while the root system is being rinsed. It has not been proved, that the ions in the xylem vessels can be exchanged but when exudation continues new ions will be taken from the central plasm into the xylem vessels. There must be an active mechanism which transfers the ions from the surface to the vessels. Where this system is localised is still unknown. Since in addition the value of the apparent free space is so much dependent on the method of determination and seems always the greater the thinner the roots (compare barley and wheat with bean and pea) it may be suggested that the AFS is localised at the root surface and in the cell walls of the root cortex. The exchange ions might be found in the protoplasm of the root cells.

If this assumption is right, it would mean that the plasmalemma would constitute an ion barrier between medium and xylem vessels. This barrier would in that case be easily crossed by the ions going inwards much more difficult in the outward direction (WALKER 1957). This concept may aid in explaining a number of observations which seem so diversifying on first sight. The influence of the transpiration rate on the ion transport may be pronounced, as long as the entrance of ions into the protoplasm is not the limiting factor in the chain of processes involved in the ion uptake.

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SUMMARY

Experiments are described in which it is shown that exchangeable rubidium ions in pea roots are transported to the shoot. The rate of this translocation is dependent on the transpiration rate. The exchangeable ions are released by the tissue to the xylem vessels without leaching out towards the medium. It is suggested that this indicates the presence of an ion barrier in the plasmalemma.

The apparent free space is thought to be localised at the root surface and in the cell walls of the cortex, whereas the exchangeable ions are found in the protoplasm.

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