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A LAG PHASE IN VACUOLAR RB^+ ACCUMULATION DURING THE INITIAL STAGE OF RB^+ UPTAKE BY ROOTS OF LOW-SALT BARLEY PLANTS

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SUMMARY

Kinetic evidence is presented to show that in roots of low-salt barley plants, vacuolar Rb^+ accumulation starts very slowly despite rapid filling of the cytoplasm. Further compartmentation of the cytoplasm with respect to ion transport is inferred.

1. INTRODUCTION

When low-salt barley plants are transferred to a salt solution, the cytoplasm of the root cells becomes the seat of an intensive transit of ions on their way to either the cell vacuoles or the stelar vessels. The results of kinetic studies (LÜTTGE & PALLAGHY 1972, HOOYMANS 1975) as well as cytochemical investigations (VAN IREN & VAN DER SPIEGEL 1975, STELZER, LÄUCHLI & KRAMER 1975) suggest that in this process the cytoplasm does not function as a simple homogeneous ion pool. Rather the evidence supports the view that in the cytoplasm a special compartment, presumably the endoplasmic reticulum or organelles derived from it, is involved in ion transport and establishes a close connection between the events occurring at the plasmalemma on the one hand and the processes leading to vacuolar accumulation and release to the xylem vessels on the other. Continuity of the endoplasmic reticulum with the desmotubuli (see ROBARDS 1971) may provide for an uninterrupted transport track throughout the root symplast.

This paper is concerned with the question of the moment at which vacuolar Rb^+ accumulation is initiated during the absorption of Rb^+ by low-salt barley plants. The results of the study reported here indicate that this process starts slowly, despite rapid filling of the cytoplasm. This delay is considered to lend support to the notion of a pluricompartmental cytoplasmic phase.

2. MATERIAL AND METHODS

Intact plants of barley (*Hordeum vulgare* L. cv. Effendi) were grown at 25 °C as follows. After disinfection in 1% $HgCl_2$ for one minute, the seeds were rinsed in flowing tap water for half an hour and then allowed to germinate in moist sand for 18 hours. After separation of the sand from the seeds, germination was continued on moist aseptic gauze supported by a stainless steel grid

over a solution of 2.10^{-4} M $CaSO_4$. Two days later, the seedlings were mounted in groups of 10 or 14 on small PVC grids and transferred to a dilute nutrient solution (0.24 mM $Ca(NO_3)_2$, 0.0075 mM $Ca(H_2PO_4)_2$, 0.10 mM $MgSO_4$, 0.05 mM KNO_3, 1 ml/l Hoagland A-Z solution) and a light/dark regime of 16/8. After three days, this solution was replaced by 2.10^{-4} M $CaSO_4$. On the following day, the seedlings were used for the experiment, each set of 10 or 14 seedlings being treated as a single plant. Since the experiments were performed in a greenhouse in different seasons, there was considerable variation between experiments with respect to atmospheric and light conditions. However, the temperature of the experimental solutions was maintained at 25 °C by a waterbath and the pH was kept at about 7 by the addition of 0.1 mM $Ca(HCO_3)_2$. The volume of the absorption vessels was chosen according to the expected uptake values, so that excessive depletion of the solution (i.e., to more than 15%) was avoided. Where necessary, transpiration was checked by placing a glass beaker lined with moist filter paper over the shoots. At the end of the uptake period, the roots were rinsed for one minute in flowing deionized water.

Unless otherwise stated, ^{86}Rb was added to the experimental solutions for radioassay of amounts of Rb^+ absorbed. Plant material was counted unprocessed in an automatic gamma counter. All transport rates and ion contents are expressed on the basis of fresh weight of the roots. Fluxes (φ) between compartments are denoted by the indices o (solution), c (cytoplasm), v (vacuole), and x (xylem vessels) in the usual way.

3. RESULTS AND DISCUSSION

Transfer of Rb^+ to the shoot shows a lag phase of 1 to $1^1/_2$ hours (*figs. 1A* and *2A*) although 2 to $2^1/_2$ hours elapse before the attainment of either a steady (*fig. 1A*) or a maximal rate preceding a secondary retardation (*fig. 2A*). At the same time, accumulation into the root declines to less than half the initial value and is maintained at this level during the rest of the experiment. An identical retardation is found in non-exuding decapitated plants (HOOYMANS 1968, *fig. 1*), which excludes the possibility that competition between transfer to the shoot and accumulation into the root underlies the decrease in the rate. Thus, Rb^+ saturation of the cytoplasm of the root cells and shifting of the rate-limiting step in root accumulation from the plasmalemma to the tonoplast offers the most obvious explanation of this pattern (cf. HOOYMANS 1964). It implies that after about $2^1/_2$ hours, further increase of the root Rb^+ content is due solely to steady vacuolar accumulation.

The question arises whether vacuolar accumulation sets in at a constant rate almost immediately after the start of the experiment (see dashed line in *fig. 1A*) or commences with a lag phase in the same way as upward translocation. In the former case the cytoplasmic Rb^+ content would approximate the ordinate intercept of the dashed line in *fig. 1A* (about 4.3 mmol.kg^{-1}), in the latter roughly all Rb^+ absorbed within 1 to $1^1/_2$ hours after the start of the experi-

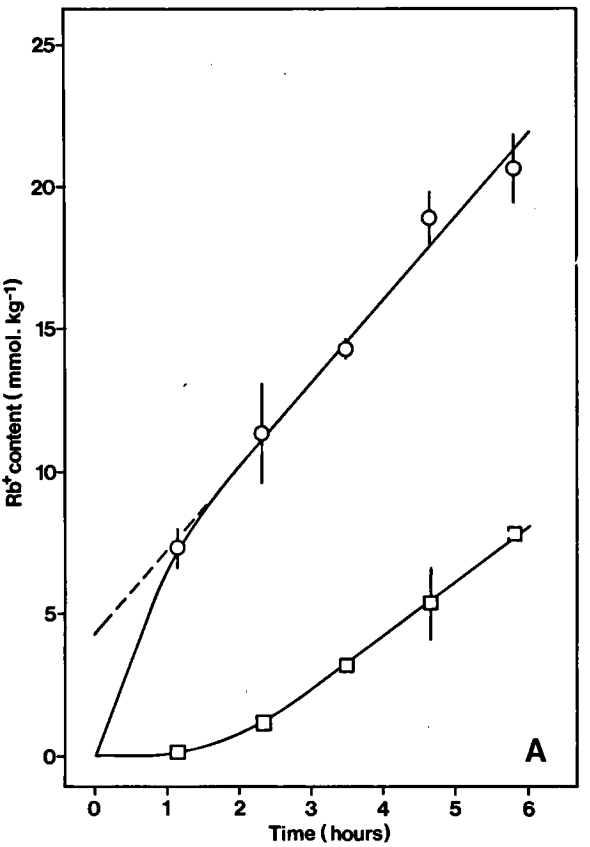
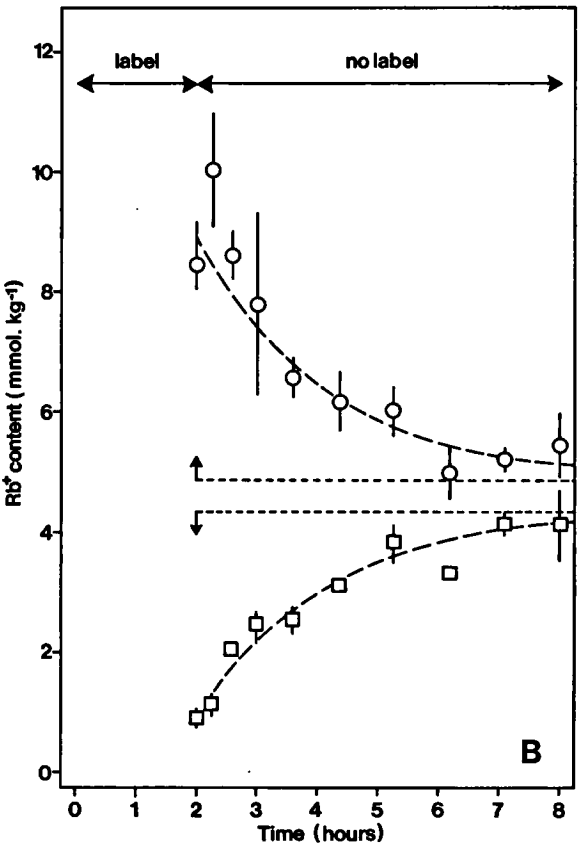


Fig. 1. A. Accumulation in the root (circles) and translocation to the shoot (squares) of Rb^+ absorbed from a solution of 0.05 mM Rb_2SO_4 + 0.5 mM $CaCl_2$. Experiment performed in duplicate (vertical bars denote lower and higher values); the dashed line extrapolates the second and linear part of the absorption curve to the ordinate.

ment (about 7.5 mmol.kg^{-1}) would be present in the cytoplasm.

When plants are allowed under the same conditions as for the experiment shown in *fig. 1A*, to absorb labelled solution during the initial two hours and are then transferred to an identical but unlabelled solution, less than half the amount of Rb^+ absorbed within the initial period is subject to upward translocation during the following six hours (*fig. 1B*). During this chase the root loses some 4 mmol.kg^{-1} of labelled Rb^+, mainly to the shoot (about 3.5 mmol.kg^{-1}) and to a small extent also to the external solution (about 0.5 mmol.kg^{-1}). The dashed lines in *fig. 1B* were fitted to the experimental points on the basis of first-order kinetics with a half-time of $1^1/_2$ hours and asymptotes as indicated by the dotted lines. The closeness of the fit indicates that there are two compartments in the root between which there is no appreciable exchange of label. In all probability, these compartments must be identified with cyto-



B. Translocation of labelled Rb^+ from the roots (circles) to the shoot (squares) in an unlabelled solution of 0.05 mM Rb_2SO_4 + 0.5 mM $CaCl_2$ after pretreatment for 2 hours in an identical labelled solution. Experiment performed in duplicate (vertical bars denote lower and higher values); dashed lines were fitted to the experimental points on the basis of first-order kinetics, and dotted lines represent their asymptotes.

plasm and vacuole in compliance with the *communis opinio*, established by the early work of BROYER (1950), that in radial ion transport the root vacuoles are by-passed. However, in redistribution experiments HOOYMANS (1974) found that all K^+ absorbed by barley plants within $7^1/_2$ hours, is eventually translocated to the shoot after transfer of the plants to a K^+-free solution. After an absorption period of this duration, an appreciable part of the root K^+ must be present in the vacuoles. To all appearance, therefore, the redistribution pattern differs fundamentally between solutions with and without K^+ (Rb^+). In any case, if it is assumed that there is virtually no lag phase in vacuolar accumulation, the results of the experiment shown in *fig. 1B* imply that during the chase almost the entire cytoplasmic pool of labelled Rb^+ (about 4.3 mmol.kg^{-1}, see *fig. 1A*) is translocated to the shoot (+ external

solution) and not to the vacuoles of the root cells. This situation is only conceivable if the cytoplasmic pool is almost completely by-passed during vacuolar accumulation. On the other hand, if vacuolar accumulation, like upward translocation, starts after a lag phase, the data in *fig. 1B* have to be explained by the distribution of a labelled cytoplasmic pool of some 8 mmol.kg^{-1} of Rb^+ over root vacuoles, shoots, and external solution in the same ratio as the fluxes φ_{cv}, φ_{cx}, and φ_{co}.

These alternatives were investigated in a chase experiment where the rate of upward Rb^+ translocation was either normal or reduced by lowering of the transpiration rate and concomitant substitution of SO_4^{--} for Cl^- (*fig. 2*). If the flux to the vacuole actually by-passes the cytoplasmic ion pool to a con-

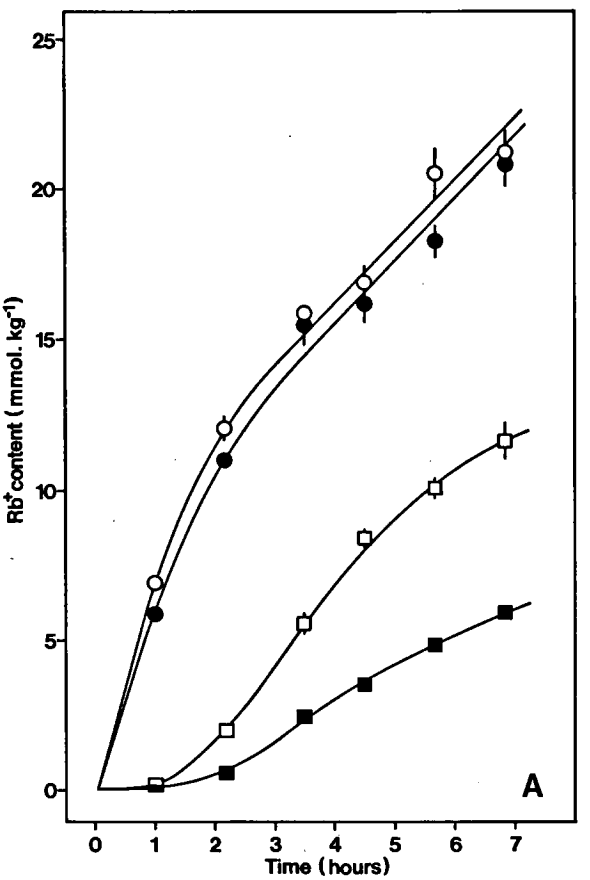
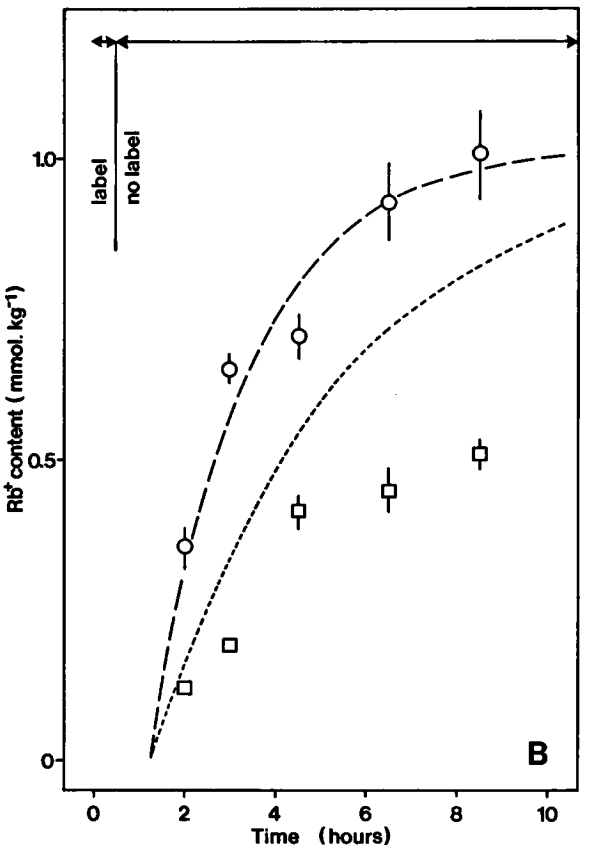


Fig. 2. A. Accumulation in the root (circles) and translocation to the shoot (squares) of Rb^+ absorbed from a solution of 0.1 mM RbCl + 2 mM $CaCl_2$ under conditions of high transpiration (open symbols) and from a solution of 0.05 mM Rb_2SO_4 + 2 mM $CaSO_4$ under conditions of low transpiration (closed symbols). Each point is the mean of 6 replicates (vertical bars denote the standard error of the mean).



B. Translocation to the shoot of labelled Rb^+ after exposure of the plants for 30 minutes to a labelled solution of 0.1 mM RbCl + 0.5 mM $CaCl_2$ followed by a chase of various durations in an unlabelled solution of either 0.1 mM RbCl + 2 mM $CaCl_2$ under conditions of high transpiration (circles) or 0.05 mM Rb_2SO_4 + 2 mM $CaSO_4$ under conditions of low transpiration (squares). Each point is the mean of 6 replicates (vertical bars denote the standard error of the mean); the dashed line was fitted to the experimental points (circles) on the basis of first-order kinetics; the dotted line represents the relation to be expected if the half-time is doubled from $1^1/_2$ hour (dashed line) to 3 hours.

siderable extent, all label present in this pool can be expected to be eventually chased to the shoot, irrespective of the rate of upward translocation; only the half-time will increase with decreasing translocation rate. Even $7^1/_2$ hours after the onset of upward transport (*fig. 2B*), the amount of label present in the shoot under the conditions of a low translocation rate is still only half that occurring under the conditions of rapid upward transfer. In *fig. 2B* the experimental values for the latter have been fitted to first-order kinetics with a half-time of about $1^1/_2$ hour and an asymptote at somewhat more than 1 mmol. kg^{-1} (see dashed line; the slightly sigmoid shape of the relation between up-

ward Rb^+ transport and time, evident from *fig. 2A*, was not taken into account in the calculations). Since the high and low translocation rates differ by a factor of approximately 2 (*fig. 2A*), it is clear that if the vacuolar flux bypasses the cytoplasmic pool, the half-time under the conditions of a low translocation rate would double from $1^1/_2$ to 3 hours at unaltered value of the asymptote (see dotted line in *fig. 2B*). Thus, after $7^1/_2$ hours the ratio between the amounts of label translocated under both conditions would approximate $(1-2^{-5})/(1-2^{-2\cdot5}) = 1.18$ instead of the experimental value of about 2. Therefore, this arrangement of the fluxes is unlikely. On the other hand, if a cytoplasmic pool of some 8 mmol.kg^{-1} of labelled Rb^+ becomes distributed over root vacuoles, shoots and external solution according to the flux ratio φ_{cv}: φ_{cx}: φ_{co} (about 2.9 : 1.9 : 0.3; see *fig. 1A*), some 3 mmol.kg^{-1} of labelled Rb^+ would eventually reach the shoot, which is in reasonable agreement with the observations (*fig. 1B*). This means that the results under discussion do not support the proposition that vacuolar accumulation proceeds at a constant high rate from all but the start of the experiment.

Admittedly, on the basis of the same data an equally strong case cannot be made against a more gradual rise in the rate of this process. More conclusive evidence on this point is provided by two experiments in which the plants were allowed to absorb labelled Rb^+ for different periods up to an hour at the start of the experiment and were then exposed to the same unlabelled solution for 7 hours. The amount of label translocated to the shoot within this period (= 3 to 4 half-times) proves to be a constant fraction of the amount of label present in root and shoot together, irrespective of the length of the labelling period (*table 1*). This finding strongly indicates that during the lag phase of upward translocation (about an hour in these experiments) either no label or no substantial amount is transferred from the cytoplasm to the vacuoles of the root cells. To illustrate this point, let us assume first that the vacuolar flux attains its maximal value within a few minutes after the start of the experiment. The relation between the specific activity of Rb^+ in the cytoplasm at the start of upward translocation at time = t_e and the duration of the labelling period (= t_o) will then be given by:

$$\log s_o/s_e = [\varphi_{oc}/(\varphi_{oc} - \varphi_{cv})] . \log t_e/t_o, \qquad (1)$$

in which s_o, s_e is the specific activity in the cytoplasm at time t_o, t_e. The flux values in these experiments are: φ_{oc} during the first hour = 4.73 mmol.kg^{-1}.h^{-1} and steady vacuolar accumulation = φ_{cv} = 1.48 mmol.kg^{-1}.h^{-1}; the mean efflux is estimated to be about 0.6 mmol.kg^{-1}.h^{-1} but is in all probability much slower during the initial period and can therefore be neglected. Starting from the finding that after a one-hour labelling period 28.1 % of the label reaches the shoot (see *table 1*), the corresponding percentages (= P) for shorter labelling periods can be computed from the formula:

$$P = [(t_e/t_o)/(s_o/s_e)] \times 28.1. \qquad (2)$$

It is clear from *table 1* that the deviations from the observed values are suf-

Table 1. Amounts of ^{86}Rb transferred to the shoot in relation to the duration of the labelling period. Intact plants were allowed to absorb labelled Rb^+ at the start of the experiment for periods of increasing duration (column 1) from a solution of 0.2 mM RbCl + 0.5 mM $CaCl_2$ before being transferred to an identical but unlabelled solution for 7 hours.* The amounts of label present in the shoot after the chase are expressed as percentages of the total amount of label present in the plant at this time (column 2). Each value is the mean of 12 (= 2 × 6) replicates.

Duration of labelling period (min)	Percentage of label present in the shoot 7 hours after end of labelling period	S.E.M.	Theoretical percentages under two assumptions:	
			φ_{cv} constant with time throughout	φ_{cv} initially proportional to time
(1)	(2)	(3)	(4)	(5)
12	26.8	0.7	13.5	24.5
24	28.8	0.7	18.5	25.3
36	27.7	0.7	22.3	26.2
48	29.0	0.7	25.5	27.0
60	28.1	0.9	28.1	28.1

* Thus, for technical reasons, the effective chase period was 48 (36, 24, 12) minutes less for plants labelled for 12 (24, 36, 48) minutes than for those labelled for an hour; however, the effect of this technical imperfection only strengthens the argument.

ficiently large to reject the hypothesis, which means that the earlier conclusions are confirmed.

To test the supposition that the flux at the tonoplast rises more slowly and gradually, it was assumed that during the initial period (= t_e) the relation between vacuolar flux (= $\varphi_{cv(t)}$) and time is given by:

$$\varphi_{cv(t)} = (t/t_e).\varphi_{cv}. \tag{3}$$

In that case the relation between s_e and s_o can be represented by:

$$s_o/s_e = [2\varphi_{oc}(t_e/t_o) - \varphi_{cv}]/(2\varphi_{oc} - \varphi_{cv}). \tag{4}$$

The percentages calculated with this formula are also given in *table 1*. The discrepancy between the theoretical and experimental values is smaller under this hypothesis, but statistical analysis – including linear transformation of equations (2) + (3) + (4) to:

$$P = [t_e(2\varphi_{oc} - \varphi_{cv})/(2\varphi_{oc}t_e - \varphi_{cv}t_o)] \times 28.1 \tag{5}$$

and testing of the regression line for passage through the origin – shows that the hypothesis can be rejected with a probability of about 400 to 1. Thus, it may be concluded that vacuolar accumulation starts considerably more slowly than proportionally to time, as assumed above. For the sake of completeness, it should be mentioned that the data provide no statistical evidence in support of the proposition that the rate of vacuolar accumulation is not nil during the initial period.

4. CONCLUDING REMARKS

From the present results it may be concluded that, despite the rapid rise of the cytoplasmic Rb^+ content at the beginning of uptake in these experiments, vacuolar accumulation starts very slowly. For instance, if the cytoplasm makes up 5% of the total tissue volume, cytoplasmic Rb^+ would attain a mean concentration of about 30 mM after 15 minutes in the experiment shown in *fig. 1A* but the rate of vacuolar accumulation at that moment would in any case be less – and probably considerably less – than $^1/_4$ of its value after an hour. Therefore, if the bulk of the cytoplasmic Rb^+ were the direct substrate for the transport mechanism in the tonoplast, the rate of vacuolar accumulation would have to rise much more sharply than just proportionally with the cytoplasmic Rb^+ content. Though the exact relation between these quantities is far from understood, it has been established repeatedly in this laboratory (cf. HOOYMANS 1964, *fig. 19*; Bange, unpublished results) that in excised barley roots at low Rb^+ concentrations the initial rate of Rb^+ uptake, and thus the cytoplasmic Rb^+ content, can be severely reduced by external Ca^{++} without a comparable effect on Rb^+ transport during the second phase, i.e., vacuolar accumulation. This divergence is directly at variance with the existence of the postulated relation. Likewise, the absence of a simple relation between the cytoplasmic Na^+ content and the rate of vacuolar Na^+ accumulation was recently inferred by HOOYMANS (1975). It seems more likely that the lag phase in vacuolar accumulation, like the delay in upward translocation, reflects further compartmentation of the ions in the cytoplasm. If, as mentioned in the introduction, special transport organelles play a role in this phase, it becomes conceivable that ions accumulated into these organelles at the plasmalemma, are delivered successively to different compartments in the root (bulk of the cytoplasm, cell vacuoles, and xylem vessels).

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