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Effects of high concentrations of sodium chloride and polyethylene glycol on the growth and ion absorption in plants

II. Multi-compartment transport box experiment with excised roots of barley

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Summary The effects of high concentrations of sodium chloride (NaCl) and polyethylene glycol (PEG) on the absorption and translocation of K and P were examined using a multi-compartment transport box with excised roots of barley. The results were as follows:

When no Ca was added, a high concentration of NaCl inhibited the absorption and translocation of K and P, although the inhibition of K was more pronounced as compared with that of P. The inhibitory effect of PEG was smaller than that of NaCl. On the other hand, the drastic inhibition of ion absorption by a high concentration of NaCl was recovered in the presence of Ca. Especially, the absorption and translocation of P was increased dramatically up to the control level by Ca, even in a high NaCl condition.

The results, especially in the presence of Ca, are quite consistent with water culture experiments in the preceding paper[15], which reported a less inhibitory effect of salt and water stresses on P absorption.

Introduction

Many studies of the various aspects of salt and water stresses on plants have been done. In the preceding paper[15], plant growth and ion absorption were examined in water culture experiments under conditions of the same osmotic potentials due to sodium chloride (NaCl) and polyethylene glycol (PEG). From the results of the experiments, it was found that NaCl inhibited more drastically cation absorption by plants than did PEG, while the inhibitory effect of both NaCl and PEG were very small on P absorption. Similar results were also obtained in the short term absorption experiments using a tracer technique.

To investigate in more detail the effects of salt and water stresses on cation and anion absorption by plants, a further experiment was undertaken to examine the absorption and translocation of K and P in excised plant roots under the same osmotic potential conditions due to NaCl and PEG. A transport box has been used to observe the translocation of ions and other substances in excised plant roots by several investigators[8,19,21,22]. In the present investigation, a "multi-compartment transport box", a modification of the transport box mentioned above, was used with the excised roots of young barley seedlings.

Materials and methods

Excised roots of 4-day-old barley (*Hordeum vulgare* L., cv. Akashinriki) were used in this investigation. Seeds of barley were allowed to germinate for 24 h in aerated water. The germinating seeds were spread on a layer of plastic screen, and grown with 0.25 m*M* $CaSO_4$ for 48 h and then with a nutrient solution for 24 h in the dark at 25°C under continuous aeration. Composition of the nutrient solution used was as follows: KNO_3 4.0 m*M*, $NH_4H_2PO_4$ 1.0 m*M*, $CaCl_2$ 1.0 m*M*, $MgSO_4$ 1.0 m*M*, Fe 1.0 ppm (as Fe-citrate), B 0.5 ppm (as H_3BO_3), Mn 0.5 ppm (as $MnCl_2$), Zn 0.05 ppm (as $ZnSO_4$), Cu 0.02 ppm (as $CuSO_4$) and Mo 0.01 ppm (as $(NH_4)_6Mo_7O_{24}$). The roots of the seedlings were excised, washed thoroughly with deionized water and used for transport experiments.

In this investigation, a specially designed plexiglass apparatus was used to examine the absorption and translocation of ions in excised roots. The apparatus is diagrammatically shown in Fig. 1, and referred to as a "multi-compartment transport box" in this paper. The apparatus consists of 4 compartments, each of which is about 10 mm long and 50 mm wide with a depth of 15 mm, with plexiglass barriers between compartments. Roman numerals in the figure indicate the positions of each compartment.

Excised roots of barley were set horizontally on the lower halves of the barriers so that the apical part of the root was put in compartment I and the basal cut end in compartment IV (see Fig. 1). Then, each upper half of the barriers was put on the roots without crushing them. The barriers were sealed with vaseline to prevent leakage of the test solution. In all of the experiments, 8 roots were used for each treatment.

^{86}Rb was used to label for potassium, and ^{32}P was used for phosphorus of test solutions. Compartment I was supplied with a radioisotope-labelled test solution, and compartments II, III and IV were supplied with non-labelled test solutions having the same chemical composition as that of the radioisotope-labelled test solution. The absorption period was about 16 h.

The osmotic potentials of both test solutions containing 65 m*M* NaCl and 60 m*M* PEG were the same (−0.3 M Pa), while the osmotic potentials of solutions without NaCl and PEG, *i.e.* the control solution, were −0.02 M Pa. The pH of all the test solutions was checked and adjusted to 4.8–4.9, especially to avoid the effect of low pH in the PEG-treated solution. The amount of NaOH added to adjust solution pH was negligible (below 0.16 m*M* as a final concentration) even in PEG-treated solution.

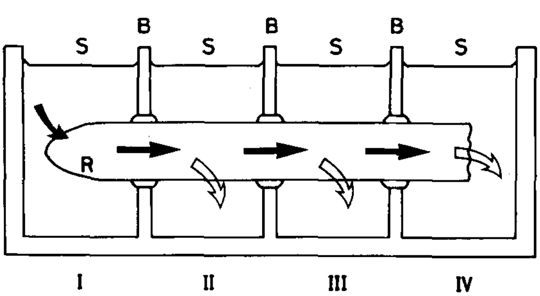

Fig. 1. Schematic representation of multi-compartment transport box. *Black arrows* show diagrammatically the absorption and translocation of ions in a plant root, and *white arrows* show the efflux of ions from a plant root. I, II, III and IV: the positions of each compartment; R: root; S: test solution; B: plexiglass barrier. For a further description, see Materials and methods.

After the absorption period, solutions in each compartment were placed in polyethylene vials for measurement by a liquid scintillation counter. The roots were cut at the barriers between each compartment and put into polyethylene vials, separately. Only the apexes of roots in compartment I, which were supplied with a radioisotope-labelled test solution, were sampled after desorption treatment with non-labelled solutions having the same chemical composition as that of test solutions for about 5 min. Radioactivity of the samples was measured by means of Cerenkov radiation[1,2,3,7,16,20] with a liquid scintillation counter. The amounts of K and P in each part of excised roots and in the solution of each compartment were calculated, based on the radioactivities of ^{86}Rb and ^{32}P respectively. The results are shown in μmoles/g fresh weight of roots/24 h. These results show the distribution of ions through the roots, and the sum of these results gives total amounts of ions absorbed in the apex of the roots.

Results

The absorption and distribution of ions in excised barley roots were examined under conditions of high concentration of NaCl and PEG in the absence and presence of Ca. The results for K and P are presented in Figs. 2 and 3, respectively. In the figures, the Roman numeral under the abscissa shows the position of each compartment in the transport box, and an asterisked numeral indicates the compartment supplied with a radioisotope-labelled test solution.

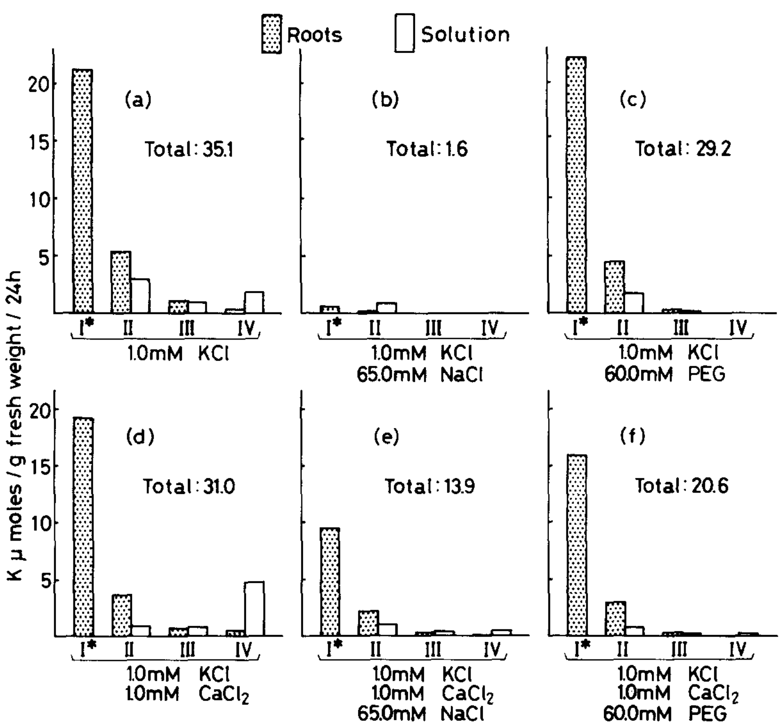

Fig. 2. Effects of NaCl and PEG on the absorption and translocation of K in excised barley roots under conditions of the absence and presence of Ca.

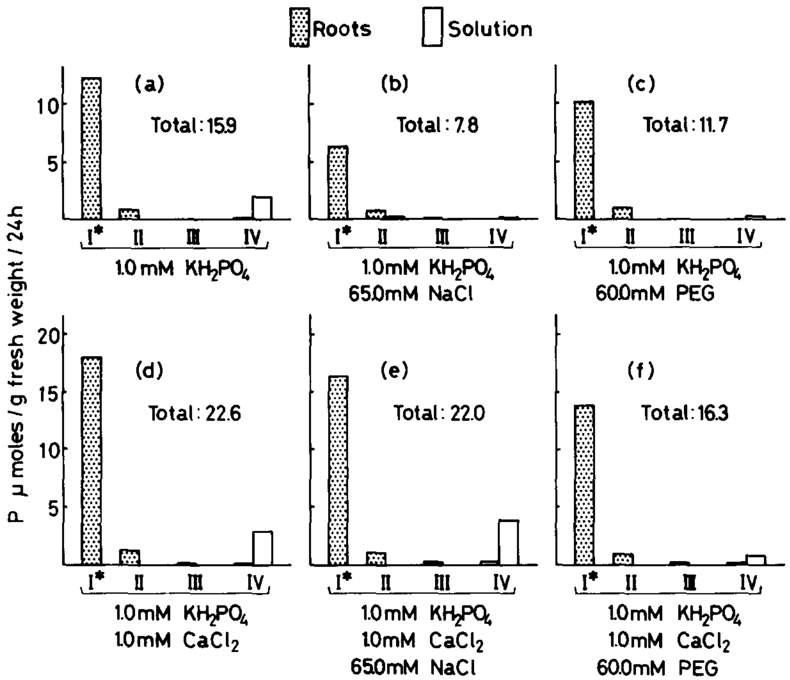

Fig. 3. Effects of NaCl and PEG on the absorption and translocation of P in excised barley roots under conditions of the absence and presence of Ca.

Experimental treatments are presented under the numerals. A shadowed bar shows the amount of ions in the roots, and an empty bar shows the amount of ions in the solution of each compartment. The total amount of ions absorbed is also presented in the figure. Results of each treatment are mean values of four to seven replications.

From Figs. 2-a, -b and -c, it is evident that a high concentration of NaCl depressed almost completely the absorption and translocation of K in the absence of Ca, while the depressing effect of a high concentration of PEG was small. On the other hand, the inhibitory effect of a high concentration of NaCl on the absorption, but not translocation, of K was recovered dramatically in the presence of Ca (Fig. 2-e), though Ca inhibited slightly the absorption of K in the control and PEG treatments (Figs. 2-d and -f).

As can be seen in Figs. 3-a, -b and -c, when no Ca was added the absorption and translocation of P were depressed in both NaCl treatment and PEG treatment, although the inhibitory effect was stronger in NaCl treatment than in PEG treatment. In the presence of Ca, total amounts of P absorbed were higher in all of the three treatments (Figs. 3-d, -e and -f), as compared with those in the absence of Ca. Especially, in NaCl treatment, the absorption and translocation of P increased up to the control level with the addition of Ca (Fig. 3-e).

Discussion

In the preceding paper[15], water culture experiments with several species of plants showed that high concentrations of NaCl and PEG depressed the absorption of cations more severely than that of P. In addition, the inhibitory effects of NaCl on ion absorption were pronounced as compared with that of PEG. To examine the absorption and translocation of K and P in the plant roots under salt and water stress conditions, multi-compartment transport box experiments were carried out with the excised roots of barley in the present investigation.

It was found that even at the same osmotic potential, a high concentration of NaCl inhibited more strongly the absorption and translocation of K than did PEG, when no Ca was added (Figs. 2-a, -b and -c). A similar relation among treatments was also shown in the absorption and translocation of P (Figs. 3-a, -b and -c), although the inhibitory effect of NaCl was more drastic for K than for P. On the other hand, the inhibition of the absorption and translocation of K and P by NaCl was recovered dramatically in the presence of Ca (Figs. 2-e and 3-e). The situation of ion absorption mentioned above, especially in the presence of Ca, is quite consistent with the results of the water culture experiments described in the preceding paper[15]. Consequently, it seems reasonable to assume that the less inhibitory effect of salt and water stresses on P absorption in water culture might be partly caused by the presence of Ca in nutrient solution.

The role of Ca in the selective absorption of monovalent cations has been the subject of intensive research, and many experimental results have been published on the stimulation of K or Rb absorption by Ca in plant roots[4,5,9,10,11,12,13,14,24,25,26]. It was reported that the stimulating effect of Ca on K absorption was more evident in the presence of Na than in the absence of it[4,10,13]. In the present investigation, it is also very clear that Ca accelerated K absorption under condition of a high concentration of NaCl (Figs. 2-b and -e). However, in the control and PEG treatment, Ca inhibited slightly the absorption of K. The results may be in some conflict with those outlined in previous papers[9,10,13,25,26]. This might be attributed to the experimental procedure used in the present investigation, in which the excised roots absorbed radioisotope-labelled ions in only 7 to 8 mm of the apex. In addition, there are some reports which showed the inhibitory effect of Ca on K absorption by bean roots[11], or in a part of the apex of the roots[24].

On the other hand, there are few reports showing the stimulating effect of Ca on P absorption in excised roots[6,17,18,23]. In the present investigation, it was clearly found that Ca accelerated the absorption and translocation of P not only in the control treatment, but also in salt and water stress treatments.

In the present investigation, excised roots of only one species, *i.e.* barley, were used because barley roots lend themselves well to use as experimental materials. It would be also interesting to examine the absorption and translocation of ions in excised roots of various plant species in addition to barley. Experimental results with some other species of plants will be presented in the following paper.

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