THE ROLE OF CALCIUM IN THE SELECTIVE CATION UPTAKE BY PLANT ROOTS

I. Effect of Cation Concentrations in Absorption Solution*

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It is well known that the selective mechanism is involved in the ion uptake process by plant roots. However, it has been very difficult to explain how living cells discriminate between such similar ions as potassium and sodium.

Viets (28) showed that the uptake of potassium and bromide by barley roots were stimulated in the presence of calcium and other polyvalent cations. The role of calcium in the ion uptake process has been studied by several groups of workers since Viets' report. On the stimulation of ion uptake by calcium, various explanations were presented in relation to *carrier* theory (4, 8, 15, 16, 18, 20, 25) or stability of *carrier* substances (5, 9, 23). The importance of calcium on the permeability of cell membranes was indicated in several experiments which were conducted by the use of chelating agents, ribonuclease and ultraviolet radiation (5, 9, 23, 24). Further, it has been noted that the effect of calcium on the ion transport is not only stimulative, but also inhibitory (6, 7, 12, 17). Recently, Jacobson et al. (13, 14), Epstein et al. (3, 22) and Waisel (29) showed that calcium was essential for integrity of the selective ion transport mechanism, and that the role of calcium was involved in the regulation of cell permeability for various ions.

In the course of studies of such calcium effect, however, some results conflicting with the report by Viets were shown on the substitution of magnesium for calcium (3, 10). On the other hand, there were some reports in which it was suggested that the calcium effect described above was not caused by calcium, but caused by accompanying anions, especially chloride (21, 26). The present investigation was initiated in order to clarify these conflicting and obscure results. Moreover, an approach was attempted to obtain further informations on the role of calcium in the selective uptake process of rubidium and sodium.

MATERIALS AND METHODS

Preparation of Root Materials

Excised roots of barlay (*Hordeum vulgare* L., variety Akashinriki) were used in this investigation. The method of culturing was similar to that described by Epstein et al. (1, 2). Seeds of barley were allowed to germinate for 24 hours in

* Main part of this paper was published in Journal of the Science of Soil and Manure, Japan, Vol. 39, p. 433–438 (1968), in Japanese.

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aerated water in the dark at 25°C. The germinating seeds were spread on a layer of plastic screen about one centimeter above the surface of 0.1 mM calcium sulfate solution. The assembly (figure 1) was placed in the dark at 25°C. The solution

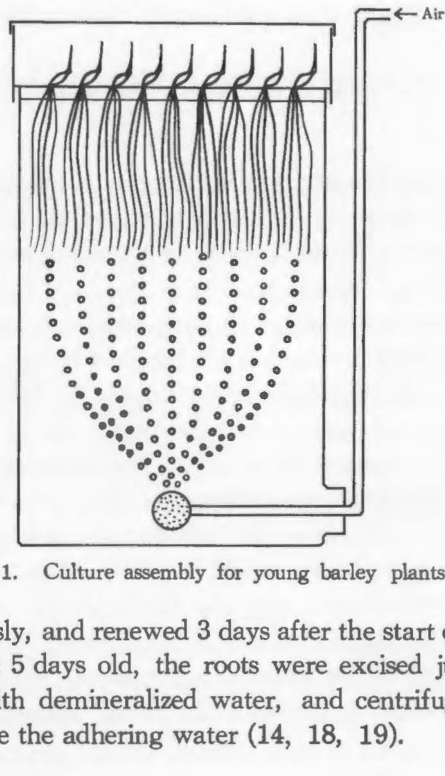

Fig. 1. Culture assembly for young barley plants.

was aerated continuously, and renewed 3 days after the start of the growth periods. When the plants were 5 days old, the roots were excised just below the screen, thoroughly washed with demineralized water, and centrifuged for 5 minutes at about $65 \times g$ to remove the adhering water (14, 18, 19).

Absorption Experiments

In order to investigate the selective uptake between rubidium and sodium, equimolar mixtures of rubidium chloride and sodium chloride were employed as absorption solutions with or without addition of calcium chloride or magnesium chloride. Initial pH of absorption solutions was adjusted to 5.5 ± 0.1. For the uptake measurements of rubidium or sodium, the absorption solutions were labeled with radioactive rubidium (^{86}Rb) or radioactive sodium (^{22}Na). In all experiments, 0.5 g of roots were used in 500 ml of absorption solution. Absorption period was 60 minutes, and the solutions were continuously aerated and kept at 25 ± 0.1°C or 2 ± 0.5°C during this time.

At the end of the absorption period, the roots were separated from the solution with a nylon mesh filter. For removing adhering solution, the roots were blotted with filter paper, shaken for 30 seconds in 30 ml of demineralized water, and this washing operation of roots was repeated once more.

Radioactive Assay

The root samples were dried, and ashed at 450°C. One ml of dilute hydrochloric acid solution was added to the ash and the samples were dried under infrared lamp. Then, the samples were counted with a Geiger-Müller counter.

RESULTS

Effect of Different Concentrations of Monovalent Cations in Absorption Solution

The uptake of rubidium and sodium by excised barley roots were investigated under wide concentration range of monovalent cations in absorption solution. The lowest concentration of monovalent cations was 0.0001 m*M* and the highest was 10.0 m*M*. Two levels of divalent cations were used at 0.05 and 0.5 m*M*.

The rate of rubidium uptake at 0.05 m*M* of divalent cations is shown in figure 2, and the rate of sodium uptake in figure 3. Besides, the rate of rubidium uptake at 0.5 m*M* of divalent cations is shown in figure 4, and the rate of sodium uptake in figure 5. Both the ordinate and the abscissa values in figures 2 to 5 are logarithmic.

As shown in figures 2 and 4, the rate of rubidium uptake represented the curves which were characterized by steep slope at lower concentration range followed by gentle slope at higher concentration range, at both 25 and 2°C; in other words, the rate of rubidium uptake was linear with increasing concentrations

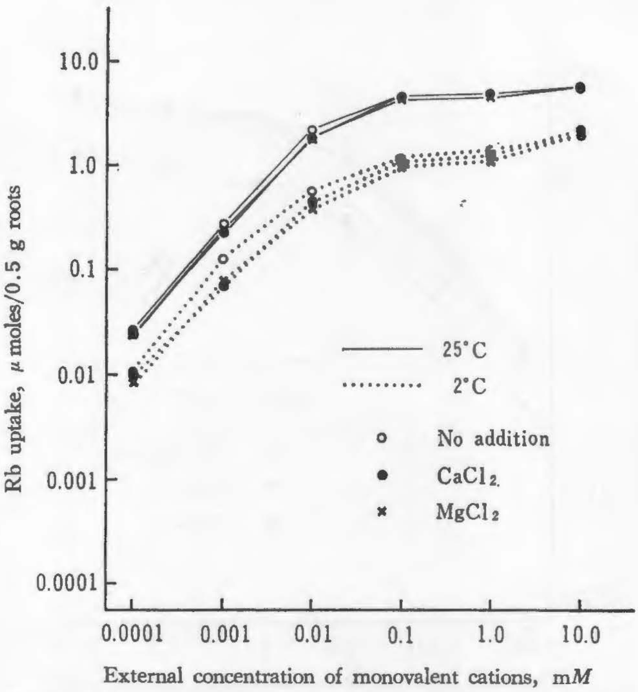

Fig. 2. Rubidium uptake in relation to external concentration of monovalent cations in the absence and presence of 0.05 m*M* of divalent cations.

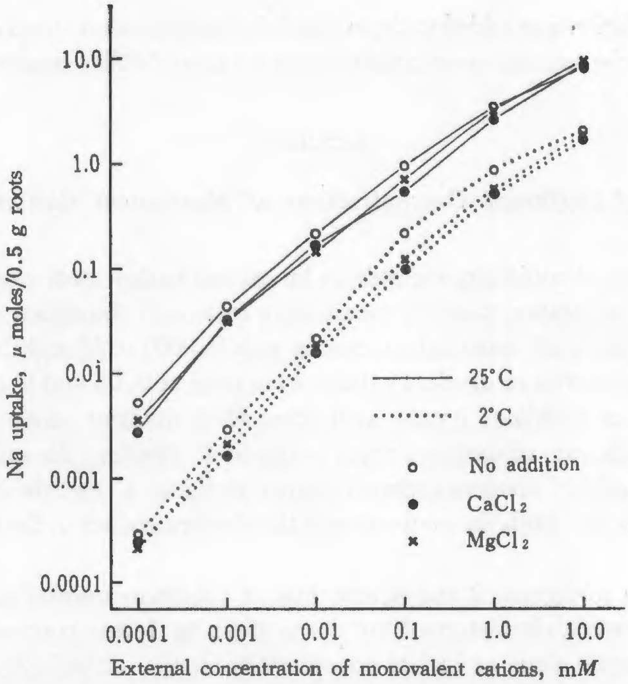

Fig. 3. Sodium uptake in relation to external concentration of monovalent cations in the absence and presence of 0.05 m*M* of divalent cations.

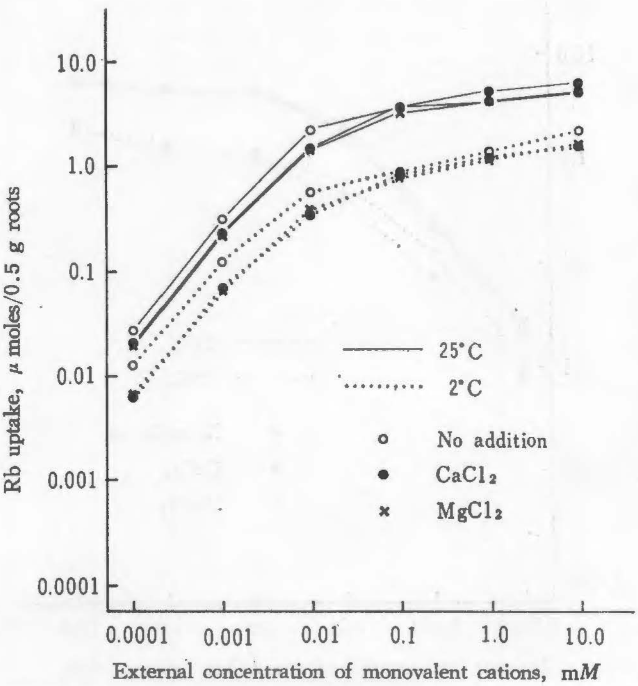

Fig. 4. Rubidium uptake in relation to external concentration of monovalent cations in the absence and presence of 0.5 m*M* of divalent cations.

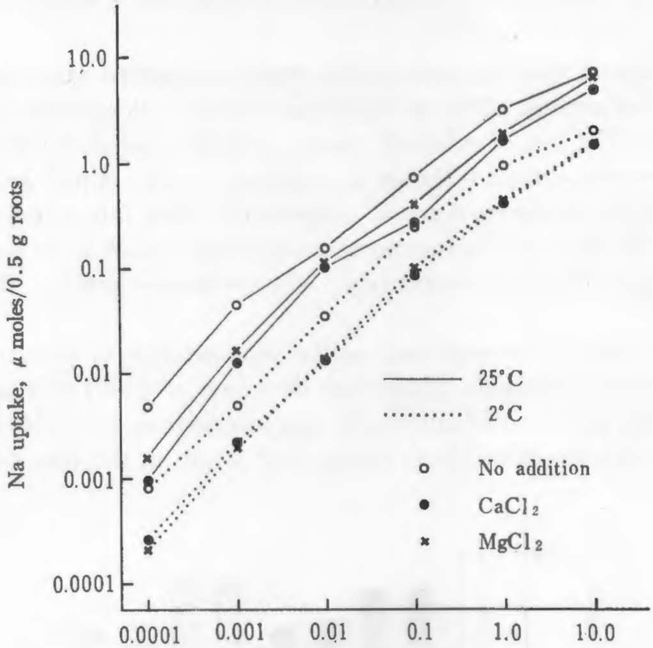

Fig. 5. Sodium uptake in relation to external concentration of monovalent cations in the absence and presence of 0.5 m*M* of divalent cations.

of monovalent cations at lower range, and then remained plateau at higher range. Hence, it was considered that rubidium uptake was dependent on the concentration in absorption solution at lower range and relatively independent at higher range. By contrast, figures 3 and 5 showed that the rate of sodium uptake represented the curves which were characterized by steep slope over all concentration range used in this experiment at both 25 and 2°C, i. e., sodium uptake was dependent on the concentration in absorption solution over all concentration range.

As can be seen through figures 2 to 5, the rate of rubidium uptake was decreased in the presence of divalent cations at lower concentration range of monovalent cations. Similarly, the rate of sodium uptake was decreased in the presence of divalent cations over all concentration range of monovalent cations. On the other hand, at higher concentration range of monovalent cations, it was likely that the rate of rubidium uptake was increased in the presence of calcium chloride at 25°C, while the rate of rubidium uptake was decreased in the presence of divalent cations at 2°C. Such effects of calcium and magnesium salts on the uptake of monovalent cations were larger at 0.5 m*M* of external concentration than at 0.05 m*M*. Further investigation was undertaken on the effect of divalent cations on rubidium and sodium uptake.

Effect of Different Concentrations of Divalent Cations in Absorption Solution

The uptake of rubidium and sodium were investigated at various concentrations of divalent cations. Two levels of monovalent cations were used at 0.001 and 1.0 m*M*. The rate of rubidium uptake at 0.001 and 1.0 m*M* of concentrations of monovalent cations is shown as percentage to the control without addition of divalent cations in figures 6 and 7, respectively. The rate of sodium uptake at 0.001 and 1.0 m*M* of concentrations of monovalent cations is shown in the same manner in figures 8 and 9, respectively. All experiments in this part were carried out at 25°C.

Figures 6 and 7 showed that, as the concentration of divalent cations increased, the rate of rubidium uptake was decreased at 0.001 m*M* of monovalent cations, but the rate of rubidium uptake was increased at 1.0 m*M* of monovalent cations. The increase of rubidium uptake was larger in the presence of calcium

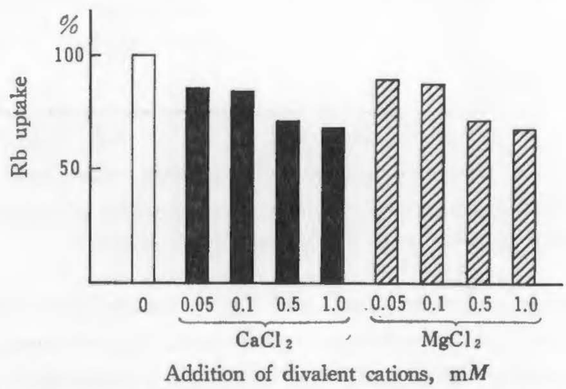

Fig. 6. Effect of various concentrations of divalent cations on rubidium uptake from absorption solutions containing 0.001 m*M* of monovalent cations, at 25°C.

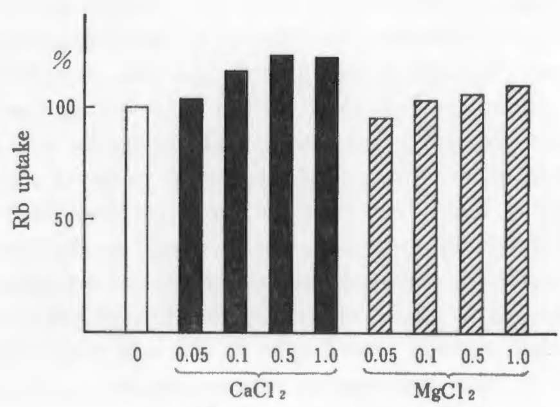

Fig. 7. Effect of various concentrations of divalent cations on rubidium uptake from absorption solutions containing 1.0 m*M* of monovalent cations, at 25°C.

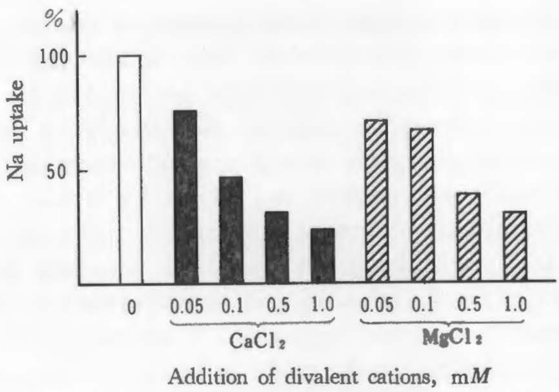

Fig. 8. Effect of various concentrations of divalent cations on sodium uptake from absorption solutions containing 0.001 m*M* of monovalent cations, at 25°C.

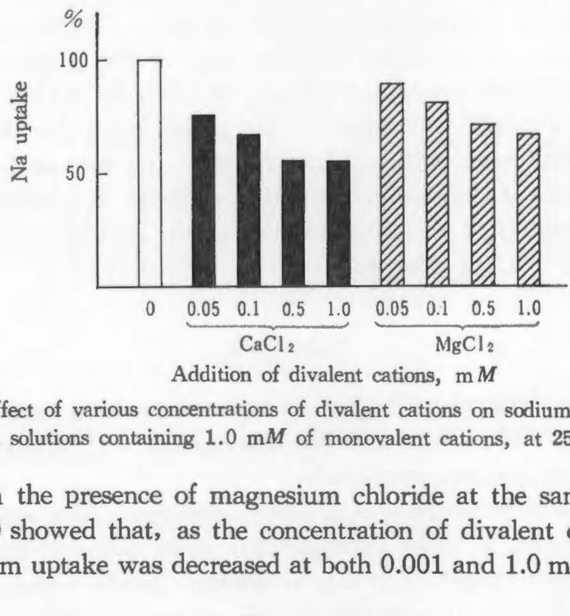

Fig. 9. Effect of various concentrations of divalent cations on sodium uptake from absorption solutions containing 1.0 m*M* of monovalent cations, at 25°C.

chloride than in the presence of magnesium chloride at the same concentration. Figures 8 and 9 showed that, as the concentration of divalent cations increased, the rate of sodium uptake was decreased at both 0.001 and 1.0 m*M* of monovalent cations.

DISCUSSION

Overstreet et al. (20) showed that calcium exerted both depressing and stimulating effect on the absorption of potassium and that the effects were related to the concentration of potassium in absorption solution. Here, it was evident that the rubidium uptake was stimulated in the presence of divalent cations at higher concentration of monovalent cations (figure 7). On the other hand, the rubidium uptake was inhibited in the presence of divalent cations at lower concentration of monovalent cations (figure 6). The sodium uptake was inhibited in the presence of divalent cations at both higher and lower concentrations of monovalent cations (figures 8 and 9). Thus, the concentration range in which the uptake of both rubidium and sodium were inhibited in the presence of divalent cations coincided with the steep slope range of monovalent cation uptake curve. The uptake of rubidium and sodium at steep slope range were governed by the concentration of monovalent cations in absorption solution. Accordingly, it was supposed that divalent cations inhibited the uptake of rubidium and sodium under such conditions that nonmetabolic factor was predominant on the ion uptake process, and that divalent cations stimulated the uptake of rubidium under such conditions that metabolic factor was predominant. A hypothesis described above was further emphasized by the fact that the stimulating effect of divalent cations on the rubidium uptake was temperature-dependent (figure 4). It was thought that the stimulating effect of calcium on rubidium uptake might be located in the metabolic process of ion uptake.

Furthermore, it was evident that magnesium chloride could be used to substitute for calcium chloride in relation to stimulating on the rubidium uptake (figure 7). However, the stimulating effect on the rubidium uptake was clearly larger in the presence of calcium chloride than in the presence of magnesium chloride at the same concentration of absorption solution. As the molar concentration of calcium chloride was equal to the molar concentration of magnesium chloride, both treatments with calcium chloride and magnesium chloride were equal in the amount of chloride in absorption solution. Accordingly, it was clear that the stimulating effect of calcium chloride on the rubidium uptake was mainly owing to calcium, but not chloride.

SUMMARY

The selective absorption of rubidium and sodium from equimolar mixture of rubidium chloride and sodium chloride was studied with relation to addition of calcium chloride or magnesium chloride.

1) The rate of rubidium uptake represented the curves which were characterized by steep slope at lower concentration range followed by gentle slope at higher concentration range. The rate of sodium uptake represented the curves which were characterized by steep slope over all concentration range used in this experiment.

2) At lower concentration of monovalent cations in absorption solution, rubidium uptake decreased in the presence of calcium chloride or magnesium chloride. Similarly, at lower and higher concentration of monovalent cations in absorption solution, sodium uptake decreased in the presence of calcium chloride or magnesium chloride.

On the other hand, at higher concentration of monovalent cations in absorption solution, rubidium uptake increased in the presence of calcium chloride or magnesium chloride. The stimulation of rubidium uptake by calcium chloride was larger than the stimulation by magnesium chloride.

3) The results obtained support that the stimulating effect of calcium chlo-

ride on rubidium uptake is caused by calcium, but not by chloride. Then, the stimulating effect of calcium on rubidium uptake could be partially substituted with magnesium chloride.

4) It was suggested that the stimulation of calcium on rubidium uptake might be located in the metabolic process of ion uptake.

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