島根大農研報 (Bull. Fac. Agr. Shimane Univ.) 24 : 233−238, 1990

An Improved Method for Measuring Ion Flow through Excised Roots and Its Application to Halophyte and Non-Halophyte Roots

Osamu HIRAYAMA*, Masashi MIHARA* and Kazuo HAYASHI*

植物切断根のイオン吸収測定の改良法ならびにその塩生植物・中生植物根への応用

平山　修・三原　真嗣・林　和夫

An improved method for measureing ion flow through excised roots is described. The excised roots were bathed in monocation solution containing 5 mM glucose (for active transport conditions) or 1 mM KCN and 0.5 mM vanadate (for passive transport conditions). The ion uptake was measured under applied pressure of 110 cm H_2O using ion chromatography. The ion transport rates were expressed on the basis of root surface area which was estimated from salt adhesion data. The present method was successfully applied to non-halophyte and halophyte roots. The results showed that the ion flow rates were much higher in halophyte roots than in non-halophyte ones. Halophyte roots (samphire, saltwort and reed) absorbed K^+ and Na^+ at a similar rate, while non-halophyte roots (spinach and cucumber) showed a selective uptake of K^+.

INTRODUCTION

Salt uptake of plant roots is closely related to the root surface and the root structure of tissues. The ion transport in the roots has been measured with radioactive ions,[1)] plexiglass transport chambers[2,3)] or perspex pressure chamber.[4)] However, there are some drawbaks in using radioactive ions or elaborate chambers. Furthermore, in order to determine characteristics of solute and water uptake in the roots, the rate of ion uptake should be expressed on a root surface area basis. The determination of the root surface area, however, is a difficult task, especially with roots with long and tangled root hairs. Thus, the root hairs are often not included in reported values of root surface areas,[4)] or the root surface area is expressed on a per weight basis.[5.6)] Recently, an improved method for determining the whole surface area of fine tomato roots was reported,[7)] but this method is still complex and time consuming.

This paper describes an improved method for measuring the ion transport rate from root to shoot using a simple apparatus under two conditions, namely, active and

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*Laboratory of Food Chemistry, Faculty of Agriculture, Shimane University, Matsue 690, Japan.

passive ion transport. The transport rates were expressed on a basis of root surface area, which was quantitatively estimated from salt adhesion data. The present method for ion flow rates was also applied to examine the ion transport properties of halophyte and non-halophyte roots.

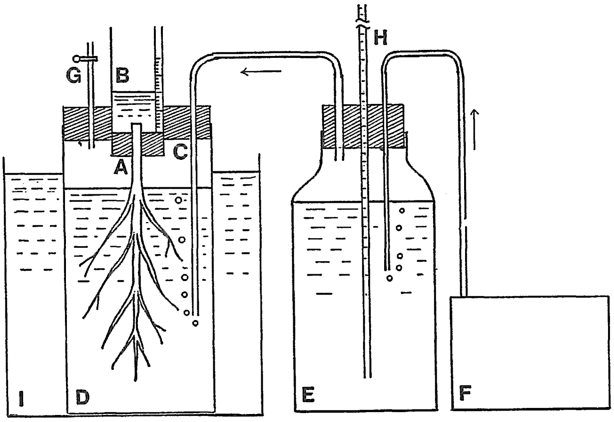
MATERIALS AND METHODS

Plant materials

Six species of plants, from halophytes to salt-sensitive species, were selected and used. Halophytes, samphire (*Salicornia europaea* L.), orach (*Atriplex Gmelinic* C. A. Mey), saltwort (*Salsola komarovi* Iljin) and reed (*Phragmites communis Trinius*) were harvested in salt farm ruins and beachs in Japan Sea. Less salt-resistant plants, spinach (*Spinacia oleracea* L.) was obtained at a market and tomato (*Lycopersicum esculentum* Mill.) was grown in a greenhouse. Salt-sensitive cucumber (*Cucumis sativus* L.) was also grown in a greenhouse.

Pretreatment

Fresh whole plants were rinsed with 0.2 mM $CaCl_2$ solution and the shoots were cut off. The excised roots were soaked in 0.2 mM $CaCl_2$ solution for 2 h with air bubbling, and washed with the bathing solutions described below.



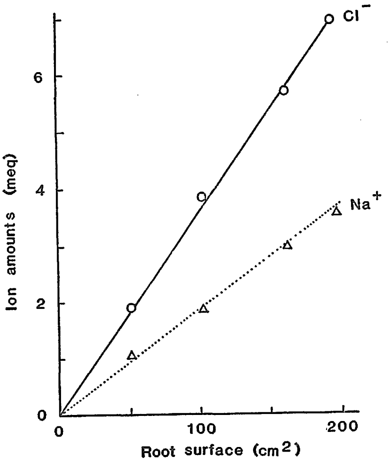


Fig. 1. Apparatus used for ion transport experiments. An excised root was fixed tightly with silicone grease in a split rubber stopper (A), which was inserted in a glass tube (B) with a measure. The tube B was then fixed through a rubber stopper (C) in a glass bottle (D). The stopper C has an inlet and outlet of compressed air, which was supplied through a bottle (E) by an air pump (F). The air pressure was adjusted by a screw clamp (G) of the outlet tube, according to the height of water level in a glass tube (H). The excised root was bathed in an ion-containing solution (see text) maintained at 25°C in a water bath (I) under pressure of 110cm H_2O. Ion and water flow into the glass tube B was followed at intervals of 1 h.

Fig. 2. Calibration curves for root surface. A Japanese radish root was bathed in 1 M NaCl solution, then freed from the excess NaCl solution, and bathed in distilled water to dissolve NaCl on the root surface. The amounts of Na^+ and Cl^- were estimated by ion electrodes, and plotted versus the determined surface area of the root.

Ion uptake experiments

The pretreated excised root was fixed in an apparatus as shown in Fig. 1, and bathed in a solution containing sampled ions at 25°C under pressure of 110 cm H_2O with air bubbling. The cut end of the root was covered with an aliquot of 1 mM Mes buffer (pH 6.8) containing 0.2 mM $CaCl_2$, and the volume and ion concentrations of the exudate were followed at intervals of 1 h. The ion concentrations were analyzed by ion chromatography, using Shimazu High Performance Ion Chromatograph HIC-6A equipped with Shin-pack IC-Cl column with 5 mM nitric acid as mobile phase.

For the root-bathing solution were used two kinds of solution: (a) solution (for active transport condition) containing 1 mM Mes (pH 6.8), 0.2 mM $CaCl_2$ and 5 mM glucose with an equimolar mixture of 10 mM of Li^+, Na^+, K^+, and Rb^+ in final concentrations, (b) solution (for passive transport condition) containing 1 mM Mes buffer (pH 6.8), 0.2 mM $CaCl_2$, 1 mM KCN, and 0.5 mM Na_3VO_4 with the same equimolar mixture of monocations as described above. The KCN and Na_3VO_4 were used as a respiration inhibitor and a plasma membrane ATPase inhibitor, respectively.

Net ion transport rates through the excised roots were calculated as follows:

$$\text{Rate of ion transport} = \frac{M_2 - M_1}{As}$$

, where M_1 and M_2 are ion contents (meq) in the exudate at time t_1 and t_2, respectively. As is surface area (cm^2) of the root.

Determination of the root surface area

The surface area of whole intact root with hairs was determined by estimating NaCl amount adhered to the root surface as follows. After the ion transport experiment, the root used was washed with distilled water, and bathed in 1 M NaCl solution for 5 min. The root was removed and washed with distilled water by bathing it for 20 s. After the excess water was removed, the root was bathed again in 1 M NaCl for 20 s, and drown up from the solution. The excess NaCl solution on the root surface was fully removed by shaking it vigorously. The treated root was then bathed in 50 ml of distilled water and gently shaked for 20 s to dissolve salt on the root surface. The amount of Na^+ and Cl^- released into distilled water was estimated with ion electrodes. Calibration curves were prepared on the basis of Na^+ or Cl^- amount per unit surface area of a Japanese radish root.

RESULTS AND DISCUSSION

Fig. 2 shows calibration curves for the root surface obtained from a Japanese radish

Table I. Determination of the surface area of the plant roots by an improved method described in text. Data are means±SE (n= 3).

Roots Fr	esh weight(g)	Surface area (cm^2)	Surfac/Fresh weight(cm^2g^{-1})
Cucumber	8.10	1190±90	147±11
Spinach	2.11	230±12	109± 6
Orach	3.40	409±10	120± 3

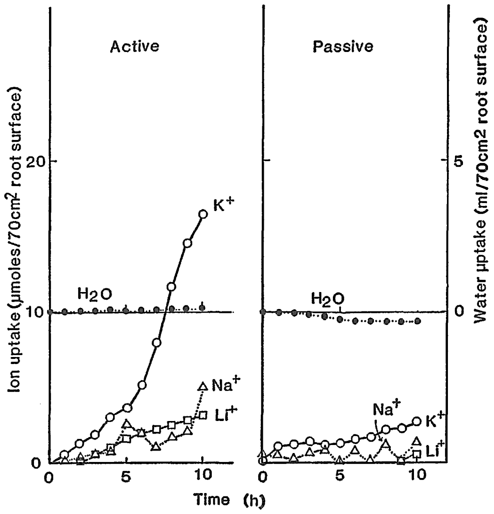


Fig. 3. Ion and water transport through excised root of non-halophyte spinach. The excised root was bathed in an equimolar mixture solution of 10 mM of Li^+, Na^+, K^+, and Rb^+, and inflow of ions and water was followed under both conditions of active and passive transport and under slight applied pressure as descibed in text.

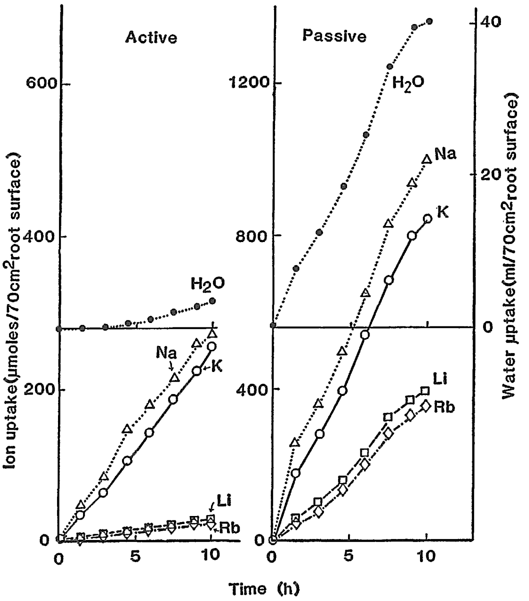


Fig. 5. Same as for Fig. 3 but for halophyte samphire.

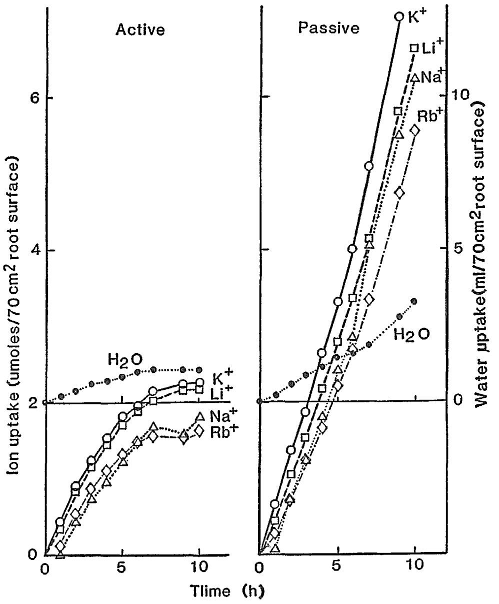


Fig. 4. Same as for Fig. 3 but for non-halophyte tomato.

root as described in Methods. The amount of Cl^- per unit of root surface area was always higher than that of Na^+, suggesting that some of the Na^+ was absorbed irreversibly on the root surface having negative charges. Thus, it seems favorable to use Cl^- rather than Na^+ for the calibration, so Cl^- curve was employed in all the experiments below.

Table I shows some example of the determination of root surface areas and surface area/weight rates. The rates of surface area/weight were at a some extent different among plant species, this reflecting the difference in shape of whole intact roots with hairs and the difference in the secrface structure of the roots.

Figs. 3, 4, and 5 show the patterns of ion and water transport in the roots of spinach, tomato, and samphire, respectively. Under the active transport conditions, under which the root respiration was stimulated with glucose and air

Table II. Ion transport rates in non-halophyte and halophyte roots. Experiments were carried out under active and passive transport conditions as described in text. The data were calculated from average values for 5 or 10 h on the basis of root surface area.

Plant roots	Active condition		Passive condition	
	K^+	Na^+	K^+	Na^+
	nmoles/cm^2 root surface·h			
Cucumber	2.2	0.6	0.8	0.4
Tomato	3.3	2.6	12	8.9
Spinach	24	7.1	3.7	5.3
Reed	18	21	17	11
Saltwort	57	65	97	124
Orach	114	114	56	50
Samphire	364	379	1214	1429

bubbling, spinach roots showed a selective absorption of K^+ from an equimolar mixture of monocations, although water uptake hardly occurred. In the presence of both inhibitors of the respiration and plasma-membrane ATPase (under the passive conditions), however, the selective uptake of K^+ disappeared (Fig. 3). Tomato roots absorbed all cations at a nearly similar rate, and these uptake rates much increased under the passive conditions (Fig. 4). Halophyte samphire roots absorbed large amounts of K^+ and Na^+ under the active conditions, and further tremendous increases occurred under the passive conditions. These results revealed that there were great differences in the patterns of ion uptake between the active and passive transport conditions and among plant species different in salt tolerance. These suggest that the ion uptake of the roots is closely related to root respiration and plasma membrane ATPase and also that the properties of the ATPase activity and respiration are different among the plant species.

Salim and Pitman[4)] showed that the excised roots under high applied pressure lost the ion selectivity which was observed in the intact roots, and transported ion-uptake ions at a much higher rate than that of ion flow in the intact roots. The present method under pressure of 110 cm H_2O showed ion selectivity in ion-uptake, and a difference in ion-flow rates among plant species. These indicate that a low pressure such as 110 cm H_2O does not change the the intact nature of the roots.

Table II shows a comparison of the transport rates of K^+ and Na^+ in non-halophyte and halophyte plant roots, on the root surface area basis calculated from the ion adhesion data. Halophyte plant roots showed much higher values than non-halophyte ones. A selective uptake of K^+ was observed in non-halophytes of cucumber and spinach only under the active conditions. Tomato, however, always showed non-selective uptake. All halophytes tested absorbed K^+ and Na^+ at a similar rate. The ion uptake rates in reed and orach were higher under the acitive conditions than the passive conditions, while those in samphire and saltwort were much higher under the passive conditions. These results suggest that a considerable eflux of ions or suppresion of ion influx occurs in samphire, saltwort and tomato roots under the active conditions.

The rates and patterns of ion uptake as described above may reflect directly the activities and properties of ATPases acting in the roots and, further, the feature in tissue construction of the roots. Thus, the present method is useful for examining the transport properties of inorganic ions and organic solutes in the roots, and also the characteristcs in the tissue structure of various plant roots.

Acknowledgments—This work was partly supported by a Grant-in-Aid for Scientific Research (No. 60480055) from the Ministry of Education, Science and Culture of Japan.

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摘　　要

植物切断根のイオン吸収を簡易に測定する方法を開発した．茎を切り取った切断根を無機イオン溶液に浸たし，これに一定の圧力（110 cm 水柱）を加え，根の切断面から浸出してくるイオンをイオンクロマトグラフィーで経時的に分析した．イオン溶液には 5 mM グルコース を添加した場合（能動輸送条件）および 1 mM KCN・0.5 mM vanadate を加えた場合（受動輸送条件）の2条件で測定した．また，同時に，根の表面積を塩付着量から求め，イオン吸収速度を根の表面積当たりの値として表現した．この方法を塩生植物・中生植物の根に応用した結果，中生植物はイオン吸収速度が低く K^+ を優先的に吸収するのに対し，塩生植物はイオン吸収速度が高く K^+ および Na^+ を同速度で吸収することが分かった．