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Physiological Response to Salinity in Rice Plant

IV. Ion exclusion in the excised root exposed to NaCl stress with hydraulic pressure*

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Abstract : Ion exclusion in excised nodal root of rice (*Oryza sativa* L. cv. Kala-Rata 1-24) exposed to NaCl stress with hydraulic pressure was studied. The effects of collecting the exudate from the stele instead of the whole cut-surface of the root, making holes to penetrate the cell layers of the epidermis, exodermis and cortical sclerenchyma, and partial coating of root surface with epoxy resin on the ion exclusion rate were investigated under 20 mmol l^{-1} NaCl at 490 kPa. Na^+ and Cl^- concentrations of the exudate from the stele and whole cut-surface of excised sorghum and corn roots were also measured and their ion exclusion capacities were compared with rice roots. Moreover, the effects of hydraulic pressure, temperature, pH, dissolved air content and ion constituent in the solution surrounding the root, and metabolic inhibitors on the ion exclusion rate were investigated.

The exclusion rate was the highest in corn, followed by sorghum then rice. It increased when exudate was collected from the stele and decreased when cell layers from the epidermis to cortical sclerenchyma were holed. It was higher in the older tissues of the root compared with the tip. Though high pressure led to a higher exclusion rate, high temperature and high pH degraded the roots and lowered the rate. Deaerated solution also decreased the rate. The exclusion rate of different ions was in the same order as the radius of the hydrated ion, i.e. $Mg^{2+} > Ca^{2+} > Na^+ > Cl^- > K^+$. The roots treated with 0.5 mmol l^{-1} 2, 4-dinitrophenol or sodium azide showed a negative linear corelation between the flow rate of the exudate and the Na^+ exclusion rate. This suggests that the barrier against the inflow of ions broke down, consequently, that the ion selectivity of roots was lost.
Key words : Endodermis, Exodermis, Ion exclusion, Metabolic inhibitor, Rice root, Salt tolerance.

塩分濃度に対するイネの生理反応に関する研究　第4報　NaCl 溶液を加圧した場合の切断根におけるイオン排除：土屋幹夫・三宅　幸・ボニラ ピルバート・熊野誠一（岡山大学農学部）

要　旨：イネの節根（切断根）に 20 mmol l^{-1} NaCl 溶液を 490 kPa で加圧した場合のイオン排除について，切断面全体あるいは中心柱部分から出液を採取した場合，表皮から厚壁細胞層までを貫く穴を開けた場合，根の表面を部分的にエポキシ樹脂で被服した場合に得られる出液イオン濃度を測定するとともに，ソルガムおよびトウモロコシの根とのイオン排除率の比較を行った．また，加圧する圧力，根を取り巻く外液の温度，pH，溶存気体量，イオン組成および代謝阻害剤の排除率に及ぼす影響を調べた．Na^+ と Cl^- の排除率は，イネ＜ソルガム＜トウモロコシの順に高く，どの植物でも中心柱部分からの出液で高かった．また，排除率は根の基部でより高く，表皮から厚壁細胞層を貫く穴を開けると低下した．一方，排除率は高圧下で高い値を示したが，高温，高 pH では根に外観的変性が認められて低下し，また脱気した場合にも徐々に低下した．イオン別に調べた排除率は，$K^+ < Cl^- < Na^+ < Ca^{2+} < Mg^{2+}$ の順に高く，水和半径に関係していることが窺われた．また，0.5 mmol l^{-1} の 2，4-dinitrophenol あるいは NaN_3 で前処理した根では，Na^+ 排除率の低下とともに出液速度の増大が認められ，溶液流入の障壁部位が壊れた結果として，イオンの選択性が低下している可能性が推察された．
キーワード：イオン排除，イネ根，外皮，耐塩性，代謝阻害剤，内皮．

Many studies of salt tolerance show that suppression of Na^+ absorption and translocation to the shoot are important to maintain growth under saline conditions[3,8,15,18]. As for the suppression of Na^+ absorption and translocation to the shoot, the relationship between ion exclusion and ATPase activity in rice roots was reported along with an increase in Na^+ content in the presence of a metabolic inhibitor[18]. These results imply that ion exclusion is an energy-dependent process. However,

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in our previous reports[9,15], it was indicated that the ion exclusion in rice root is basically similar to the process of reverse osmosis, which is driven by pressure, and that it is a non-metabolic process. Also, it was suggested that the ion exclusion sites in root are mainly the exodermis and endodermis. In addition, we reported that Na^+ concentration in the exudate from the stele in root immersed in NaCl solution under hydraulic pressure decreased significantly[9]. In this study, we investigated the characteristics of ion exclusion of excised rice root exposed to NaCl solution with hydraulic pressure under different conditions. Moreover, the rice root ion exclusion capacity was compared with that of sorghum and corn roots, and the site of ion exclusion in root and the effect of metabolic inhibitors on exclusion were determined.

Materials and Methods

1. Varieties used, growing conditions and sampling period

In this experiment, we used one variety of rice, sorghum and corn, i.e. Kala-Rata 1-24 (KR1), Yukijirushi Hybrid and Snowdent G4513, respectively. Rice was grown in Kimura B culture solution, and sorghum and corn were grown in Kimura A culture solution in the greenhouse at Okayama University. Nodal roots of 6～8 cm in length were sampled at the 8th leaf stage for rice, and the 5th leaf stage for sorghum and corn.

2. Exudate Sampling, Ion Determination and Ion Exclusion Rate

In order to gain exudate from the cut-surface of excised nodal root immersed in NaCl solution with hydrostatic pressure, a self-made pressure device, which was composed of a nitrogen gas tank, pressure gauge and regulators, and 6 chambers, was used. The procedure of setting-up the root in the chamber was similar to our previous report[14]. A test tube (35 ml) filled with 20 mmol l^{-1} NaCl solution of 20°C was set in the chamber. The cut-surface of an excised root was put in the stainless steel pipe (1.0～3.0 mm in inner diameter, 70 mm in length), which was attached to the cap of a chamber and glued with epoxy resin. Then the cap was closed so that the root was immersed in the NaCl solution in the test tube. Pressure was slowly applied and controlled at 490 kPa by using a nitrogen gas tank and regulator and about 50 μl of the exudate from the root was gathered using a micro-syringe every 4 hours. Ion concentration in the collected exudate was measured by ion chromatography (Shimadzu HIC-6A). For the anion analysis, a mixed solution of 8 mmol l^{-1} p-hydroxybenzoic acid and 3.2 mmol l^{-1} Bis-Tris was used as mobile phase at the flow rate of 1.4 ml min^{-1}, and for the cation, 5 mmol l^{-1} oxalic acid was used at 1.5 ml min^{-1} flow rate. Ion exclusion rate was evaluated based on the relative values of ion concentrations in the exudate to that of the medium. The relative values were computed being based on the average of ion concentration per root.

3. Determination of site of ion exclusion

With regard to the site of ion exclusion, the effects of collecting the exudate from the stele with endodermis instead of the whole cut-surface of the root, partial covering of root surface with epoxy resin, and making holes so as to penetrate epidermis, exodermis and cortical sclerenchyma on ion exclusion rate were investigated under 20 mmol l^{-1} NaCl at 490 kPa.

To get the exudate from the stele with endodermis, cell layers from epidermis to lysigenous aerenchyma in the tissue of 1 cm section from the cut-surface were removed. Ion exclusion rate in the exudate from the stele with endodermis was compared with that from the whole cut-surface. Furthermore, in order to determine the contribution of the cell layers from epidermis to cortical sclerenchyma to ion exclusion, many holes penetrating the cell layers of epidermis, exodermis and cortical sclerenchyma but not injuring endodermis were made at 5 mm intervals along the root length using a glass needle of about 70 μm in diameter and about 300 μm in length under a stereoscopic microscope. Also, the ion exclusion rates of mature region and meristematic and elongating region of root were compared by completely coating with epoxy resin over the root surface of tip region from the apex to 2 cm, or over the surface of remaining region except 2 cm from apex.

4. Physiological characteristics of ion exclusion

The physiological characteristics of ion exclusion were determined under different conditions, i.e. at 20 and 40°C of solution temperature, at pH 4.0, 5.5, 7.0 and 8.5, and at 294 and 686 kPa pressures. Also, the influence of ion constituents on ion exclusion was estimated using 20 mmol l^{-1} NaCl solutions prepared by adding NaCl to either Kimura B culture solution of standard concentration or distilled water. Furthermore, the deaerated NaCl solution by ultrasonication (Branson : B72) with aspiration was also used to evaluate the influence of dissolved air on ion exclusion. To investigate the difference of ion exclusion among ion species, 20 mmol l^{-1} solutions of K^+, Ca^{2+} and Mg^{2+} were prepared by adding KCl, $CaCl_2$ or $MgCl_2$ instead of NaCl to culture solution.

5. Effect of metabolic inhibitors to ion exclusion

The ion exclusion of roots treated with metabolic inhibitors was examined to determine whether ion exclusion is an energy-dependent process or not. Ouabain, which is known as an inhibitor of Na^+, K^+-translocating ATPase and Na^+ active transport in animal cells, sodium azide (NaN_3), which is inhibitor of oxidative phosphorylation and cytochrome oxidase, and 2, 4-dinitrophenol (DNP), which is inhibitor of oxidative phosphorylation,[5,18] were used as metabolic inhibitors. Seven roots were immersed in a test tube filled with 10 ml of 0.5 mmol l^{-1} inhibitor for 1 hour, while roots for the control were immersed in distilled water. Then, roots were placed on filter paper and the remaining solution was removed from the root surface by rinsing with distilled water three times. The roots were set up in the pressure chamber and the Na^+ exclusion rates of those roots were determined. In the DNP treatment, 0.25, 0.1 and 0.05 mmol l^{-1} DNP conditions were also added.

Unless specified, experimental conditions were all at 20°C under 490 kPa, using 20 mmol l^{-1} NaCl solution of pH 5.5 for rice and pH 6.0 for sorghum and corn, and the exudates were collected from the stele with endodermis.

Results

1. Character of exudate from root exposed to NaCl stress with dydraulic pressure

Table 1 Shows the amount, pH and ion concentrations of the exudate from KR1 root exposed to the standard concentration of Kimura B culture solution containing 20 mmol l^{-1} NaCl with 490 kPa hydraulic pressure. The exudate was sampled at the rate of about 5 μl h^{-1}, from 11 hours after hydraulic pressure was applied. The average pH of exudate was about 8.4 or 2.9 higher than the pH value of the external medium. The positive and negative ionic charges, which were the sum of positive or negative ionic charges calculated as the product of molar concentration of detected ion and its ionic charge, were almost balanced. However, the epoxy resin used tested alkaline by litmus paper test and may have contributed to the increase in pH.

In terms of ion concentration, the exudates generally contained 7.9 and 9.1 mmol l^{-1} of Na^+ and Cl^-, respectively, while K^+, Ca^{2+} and $SO_4{}^{2-}$ were only about 0.44, 0.64 and 0.03 mmol l^{-1}, respectively, and the amount of $NH_4{}^+$, Mg^{2+}, $NO_2{}^-$ $NO_3{}^-$ and $H_2PO_4{}^-$ were undetected.

2. Site of ion exclusion

The Na^+ concentration in the exudate from the stele remarkably decreased (Fig. 1). This was similar to the results in sorghum and corn roots (Fig. 2). Na^+ was excluded about 49% (based on the average of Na^+ concentration) through the cut-surface or about 72% through the stele in roots without the holes, and only about 36% through the stele in the roots with the holes (Fig. 1).

Flow rate of the exudate was different for each kind of roots used. The average flow rate of exudate was 7.22, 4.87 and 18.77 μl h^{-1} for the cut-surface and the stele of roots without holes, and the stele of roots with holes, respectively. The flow rate of exudate for cut-surface with holes was unestimated because it was too fast to measure.

Results from the experiment covering the root surface of tip region from the apex to 2 cm, or the surface of remaining region except 2 cm from apex suggested that Na^+ exclusion mainly occurs in the older tissues or region of the root where early maturation in terms of Casparian strip development and early lignification occurs (Fig. 3).

3. Physiological characteristics of ion exclusion

The relationship between the flow rate and Na^+ concentration of exudate under different hydrostatic pressures was investigated. Results revealed that higher pressure led to a higher

Table 1. Amount, pH and ion concentrations of the exudate from rice root (Kala-Rata 1-24) exposed to the kimura B culture solution of standard concentration containing 20 mmoll^{-1} NaCl with hydraulic pressure of 490 kPa.

Sampling	Time (hr.)[1]	11	15	19	23	27	31	35
Amout of	1[2]	40.0	37.0	29.5	26.5	23.0	17.0	15.0
exudate	2	13.5	26.0	27.0	27.0	23.0	19.0	14.0
(μl)	3	15.0	24.0	20.0	26.0	18.0	12.0	10.0
	average	22.7	29.0	25.5	26.5	21.3	16.0	13.0
pH	1	9.0	9.2	9.1	9.0	8.8	8.4	8.1
	2	8.5	8.6	8.6	8.3	8.0	8.1	8.0
	3	7.9	8.0	8.1	8.1	8.2	8.1	8.1
	average	8.5	8.6	8.6	8.5	8.3	3.2	8.1
Na^+	1	11.2	10.0	9.5	9.8	10.8	11.7	11.4
(mmol l^{-1})	2	7.2	6.8	6.8	7.3	7.4	8.0	8.2
	3	4.7	4.7	4.8	6.5	6.5	6.7	6.7
	average	7.7	7.2	7.0	7.9	8.2	8.8	8.8
K^+	1	1.7	0	4.2	1.6	0	0	0
(mmol l^{-1})	2	0	0	0	0	0	0	0
	3	0	0	1.8	0	0	0	0
	average	0.6	0	2.0	0.5	0	0	0
Ca^{2+}	1	0	0	0	0	0.9	0.6	1.1
(mmol l^{-1})	2	0	0	0	0.6	1.2	0	1.4
	3	1.1	1.3	0	1.1	1.1	1.1	1.6
	average	0.4	0.4	0	0.6	1.1	0.6	1.4
Cl^-	1	10.4	9.7	10.7	10.5	10.5	15.0	12.4
(mmol l^{-1})	2	10.0	6.2	7.2	6.3	8.3	8.2	10.9
	3	8.2	5.2	6.3	7.5	7.8	9.2	11.5
	average	9.5	7.0	8.1	8.1	8.9	10.8	11.6
$SO_4{}^{2-}$	1	0.3	0	0	0	0.2	0	0
(mmol l^{-1})	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	average	0.1	0	0	0	0.1	0	0
Na^+	1	44.0	50.0	52.5	51.0	41.0	41.5	43.0
exculsion	2	64.0	66.0	66.0	63.5	63.0	60.0	59.0
rate (%)	3	76.5	76.5	76.0	67.5	67.5	66.5	66.5
	average	61.5	64.0	65.0	60.5	59.0	56.0	56.0
Cl^-	1	48.0	51.5	46.5	47.5	47.5	25.0	38.0
exclusion	2	50.0	69.0	64.0	68.5	58.5	59.0	45.5
rate (%)	3	59.0	74.0	68.5	62.5	61.5	54.0	42.5
	average	52.5	65.0	59.5	59.5	55.5	46.0	42.0
Ratio of	1	1.18	1.04	1.29	1.08	1.15	0.87	1.09
ionic charges	2	0.71	1.11	0.94	1.34	1.18	0.98	1.01
(+/−)	3	0.87	1.32	1.08	1.14	1.12	0.96	0.86
	average	0.92	1.16	1.10	1.19	1.15	0.94	0.99

1) First exudate was sampled at 11 hours after the start of applying 490 kPa hydraulic pressure to root, then sampling was done every four hours.

2) Sample number of root.

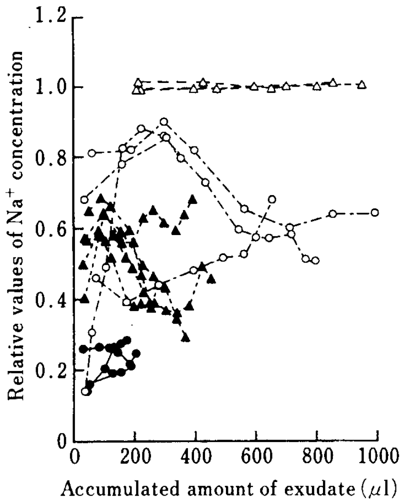

Fig. 1. Na^+ exclusion rate in excised rice root (Kala Rata 1-24) exposed to the Kimura B culture solution of standard concentration containing 20 mmol l^{-1} NaCl with hydraulic pressure of 490 kPa.
Exudate was collected from ;
▲ : the whole cut-surface of excised root without holes,
△ : the whole cut-surface of excised root of which subepidermal cortical layers were holed,
● : the stele with endodermis of excised root without holes
○ : the stele with endodermis of excised root of which subepidermal cortical layers were holed.

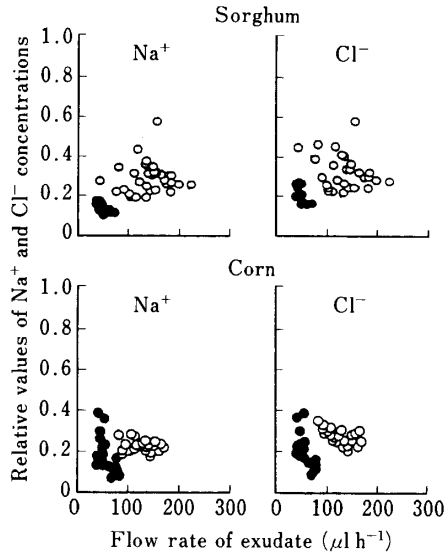

Fig. 2. Na^+ and Cl^- exclusion rates in sorghum and corn roots exposed to the Kimura B culture solution of standard concentration containing 20 mmol l^{-1} NaCl with hydraulic pressure of 490 kPa.
Exudate was collected from ;
○ : the whole cut-surface of excised root,
● : the stele with endodermis of excised root.

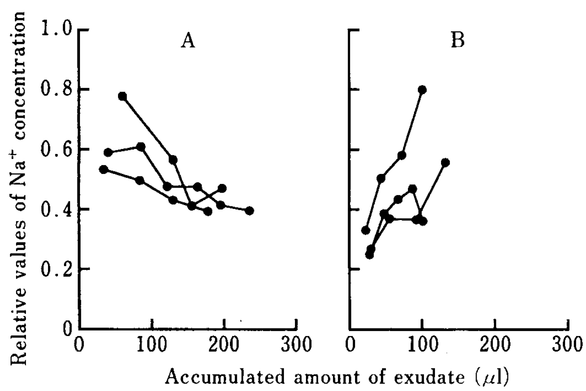

Fig. 3. Na^+ exclusion rate in the root surface of the remaining region 2 cm from apex (A) and of the tip region from the apex to 2 cm (B).

exclusion rate, i.e. the exclusion rate at the same flow rate of exudate was higher under 686 kPa than under 294 kPa (Fig. 4). This result strongly supports that Na^+ exclusion is dependent on driving pressure.

Exclusion rate at 40°C NaCl solution (Fig. 5) and at pH 7.0 and 8.5 (Fig. 6) was remarkably decreased. Based on the ocular observation of the roots subjected to these treatments, the roots turned yellow and shrank. Also, the ion exclusion rate from the exposed to deaerated solution decreased (Fig. 7).

The Na^+ and Cl^- exclusion rate in the case of using NaCl Solution adjusted in distilled water was as high as in culture solution (Fig. 8). However, the exclusion rate of different ions differed in the following order : $Mg^{2+} > Ca^{2+} > Na^+ > Cl^- > K^+$ (Fig. 9).

4. Effect of metabolic inhibitors to ion exclusion

To further investigate the relationship between ion exclusion and energy supply, roots were allowed to be permeated with three kinds of metabolic inhibitors. Results showed that Na^+ exclusion was not affected by Ouabain, but DNP and NaN_3 made the Na^+ exclusion to decrease (Fig. 10). Since with DNP, the Na^+ exclusion decreased by more than 50%, we further investigated roots permeated with four different DNP concentrations. Higher DNP concentrations resulted in a higher flow rate of exudate (Fig. 11). There was a significant relationship between the average flow rate of exudate per root and the

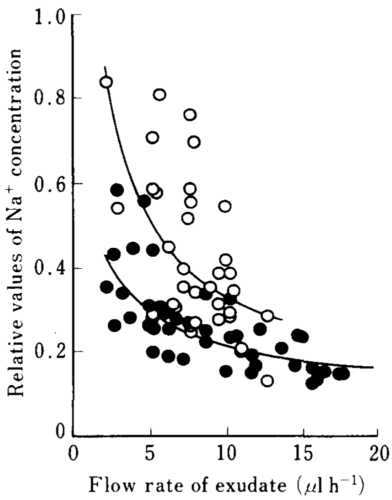

Fig. 4. Effect of applied pressure on Na^+ exclusion rate.
○ : 294 kPa, ● : 686 kPa.

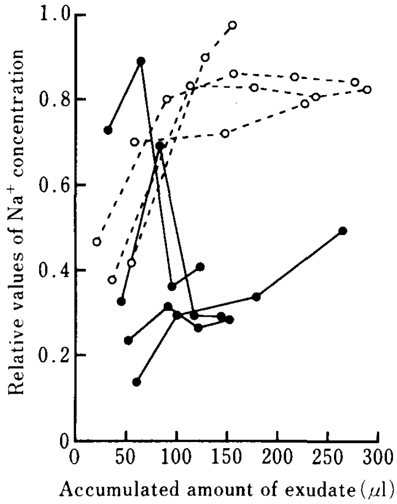

Fig. 5. Changes in Na^+ concentration of the exudate from roots exposed to 20 mmol l^{-1} NaCl under different temperatures.
●—— : 20°C, ○----- : 40°C.

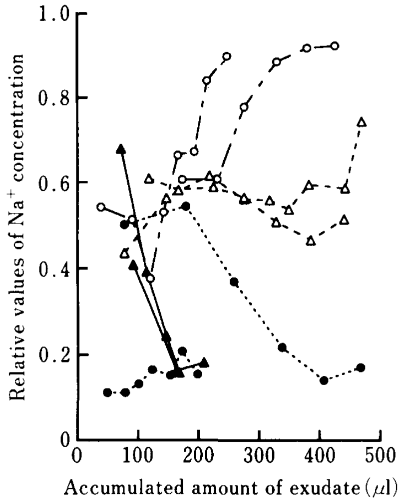

Fig. 6. Changes in Na^+ concentration of the exudate from roots exposed to 20 mmol l^{-1} NaCl under different pH levels.
▲—— : pH 4.0, ●······ : pH 5.5,
△----- : pH 7.0, ○—— : pH 8.5.

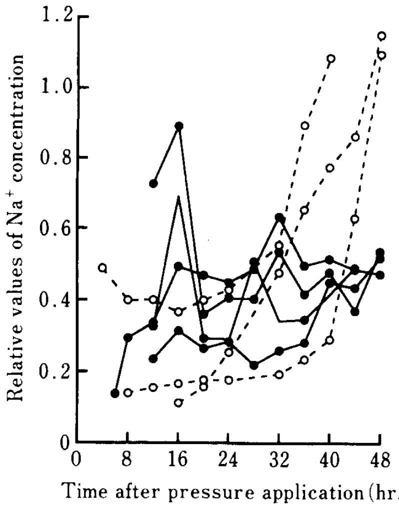

Fig. 7. Changes in Na^+ concentration of the exudate from roots exposed to deaerated 20 mmol l^{-1} NaCl.
●—— : Control, ○----- : deaerated treatment.

Na^+ concentration and higher flow rate of exudate led to lower efficiency of Na^+ exclusion (Fig. 11).

Discussion

The flow rate of the exudate at 490 kPa was about 5 μl h^{-1}. The rate is considered to be almost the same level of water flux during transpiration, judging from that of a seedling of KR1 at the 7～8 leaf stage having about 200 roots[14) transpired at the rate of 0.6～1 gH_2O per hour[15). The pH value of exudate from the stele increased to about 8.4 when that of the external medium was 5.5. The alkaline nature of the epoxy resin used may be the cause. However, there was no correlation between the pH and the Na^+ and Cl^- exclusion rate in Table 1. Also, using a heat-melt resin by the Hot Glue gun (Henkel Düsseldorf: PXP 1) Peter gun, which is not reactive to litmus paper, the average Na^+ and Cl^- exclusion rate of KR1 was 36% and 46%, respectively. These indicate occurrence of ion exclusion regardless of the glue. Changes in pH in relation to the ion exclusion with applied

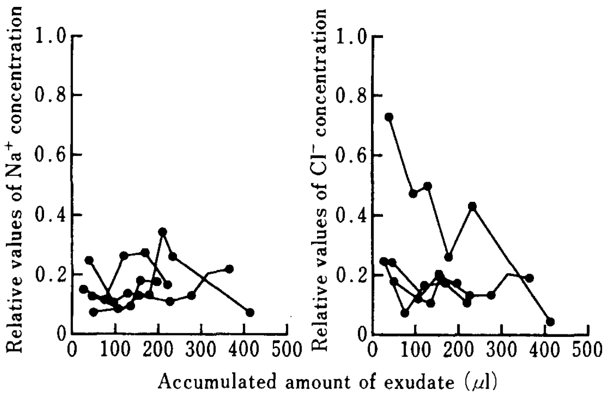

Fig. 8. Na^+ and Cl^- concentrations of the exudate from roots exposed to 20 mmol l^{-1} NaCl in distilled water.

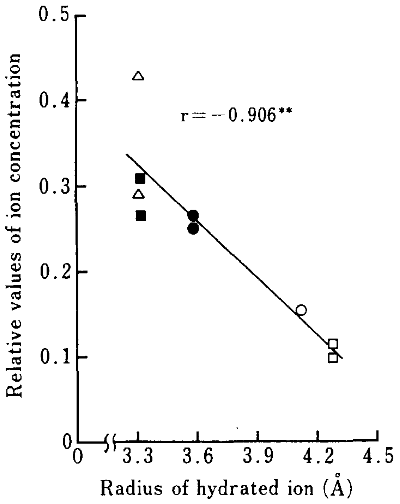

Fig. 9. Relationship between the radius of hydrated ion and the relative ion concentration of the root exudate to that of the medium. △ : K^+, ■ : Cl^-, ● : Na^+, ○ : Ca^{2+}, □ : Mg^{2+}.

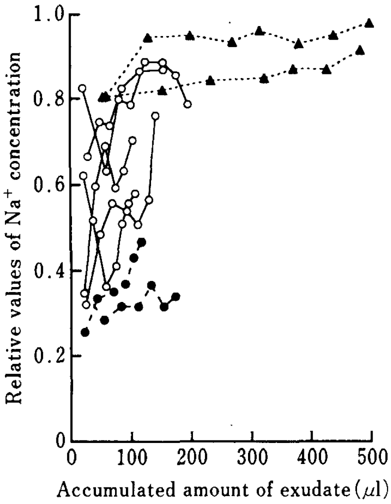

Fig. 10. Changes in ion exclusion capacity of roots treated with different metabolic inhibitors.
●----- : Ouabain, ○—— : NaN_3, ▲······ : DNP.

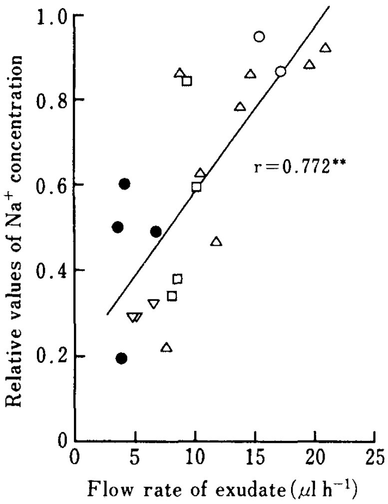

Fig. 11. Relationship between flow rate of exudate and changes in ion exclusion capacity of roots treated with different DNP concentrations.
○ : 0.5 mmol l^{-1}, □ : 0.25 mmol l^{-1}, △ : 0.1 mmol l^{-1}, ▽ : 0.05 mmol l^{-1}, ● : Control.

pressure requires further research.

The result that Na^+ concentration in the exudate from the stele of rice, soughum and corn roots remarkably decreased compared with that from the whole cut-surface implies that the cell layers from endodermis to xylem play an important role during ion exclusion. When the stele in which the cell layers from epidermis to cortical sclerenchyma were holed, the ion exclusion rate decreased from 72% to 36%. These results clearly showed that ion exclusion occurs at the cell layers of epidermis, exodermis and cortical sclerenchyma. This coincides with the result in our previous report[9].

The flow rate of water is also impeded by about 74% at the cell layers of epidermis, exodermis and cortical sclerenchyma, and 33% on the way from endodermis to xylem. It is known that developed endodermis and subepidermal cortical layers block the apoplastic movement of solution, and at these sites, nearly all of the water has to flow through cell membranes with very low permeability to ions[4,13]. From this viewpoint, one of the sites of ion exclusion could be at the plasma membrane.

The subepidermal cortical layers i.e. exodermis and cortical sclerenchyma of rice are located at the second and third layer of cells from the epidermis, respectively. In general, the exodermis resembles the endodermis histochemically and structurally in terms of presence of Casparian strip[2]. Based on the results above, there are two possible sites of ion exclusion. The outer site might be at the exodermis and cortical sclerenchyma, and the inner site at the endodermis.

The root of plants under marginal conditions, such as salinity and water stress, results in induction of lignification, suberization and appearance of Casparian bands near the root apex[1,12]. It is considered that these histochemical and structural changes contribute to the increase in the efficiency of ion exclusion. Considering reverse osmosis, the lignification and suberization of cell wall provide endurance to withstand negative pressure by making the tissues rigid.

The high temperature and pH treatments resulted in a decreased ion exclusion rate. Several reports[6,11] showed that a membrane composed of bimolecular lipid layer and sterol is considerably affected by temperature. Consequently, the structure, stability and substantial permeability of membrane are also affected by high temperature[11] and probably pH. The decrease in exclusion efficiency from the roots exposed to deaerated solution also implied that oxygen content may be related to the maintenance of the membrane structure.

The exclusion rate differed with the kind of ions in the following order: $Mg^{2+} > Ca^{2+} > Na^{+} > Cl^{-} > K^{+}$. Judging from the data that the radius of hydrated ion has also the following order i.e. 4.28, 4.12, 3.58, 3.32 and 3.31 Å[10], respectively, it is assumed that the ion exclusion rate has a close relaiton to the radius of hydrated ion. A similar relationship was also found in the investigation of the relationship between the radii of hydrated ions and transpiration stream concentration factors of the ions which were measured in intact rice plants under different conditions of relative humidity[16].

Treatment with DNP and NaN_3 resulted in a decrease of the ion exclusion rate and increase of exudate flow rate, particularly at 0.1 to 0.5 mmol l^{-1} DNP. It was considered that the metabolic inhibitor altered/damaged the barriers to ions and water movement. Consequently, it may have led the ions to pass barriers with less selectivity. Results implied that the increase in Na content with the presence of metabolic inhibitor does not always suggest the close association of an energy-dependent process for excess-ion exclusion, but may be due to the alteration caused by the metabolic inhibitor to the barrier of ion exclusion. This may explain the results observed on the leakage of solutes from soybean leaf[7] and root tissues[17] under salt stress.

Judging from the above results, the ion exclusion in rice root may basically occur at exodermis and endodermis when salt solution flows into xylem through the mature or older region of root and it doesn't depend on energy directly.

References

1. Cruz, R.T., W.R. Jordan and M.C. Drew 1991. Structural changes and associated reduction of hydraulic conductance in roots of *Sorghum bicolor* L. following exposure to water deficit. Plant Physiol. 99 : 203—212.
2. Esau, K. 1965. Plant Anatomy. Second edition. Toppan Co., Japan. 481—538.
3. Flowers, T.J. and A.R. Yeo 1989. Effects of salinity on plant growth and crop yields. In Cherry, J. H. ed., Environmental Stress in Plants. Biochemical and Physiological Mechanisms. Springer-Verlag, Berlin. 101—119.
4. Greenway, H. and R. Munns 1980. Mechanisms of salt tolerance in nonhalophytes. Ann. Rev. Plant Physiol. 31 : 149—190.
5. Kawasaki, T., S. Hori and M. Moritsugu 1970. Effect of calcium on monovalent cation absorption by crop root. (3) Effect of several metabolic inhibitors (NaCN, DNP, Ouabain). Jpn. J. Soil Sci. Plant Nutr. 41 : 467—472**.
6. Kuiper, P.J.C. 1984. Functioning of plant cell membranes under saline conditions : membrane lipid composition and ATPases. In Staples, R.C. and G.H. Toenniessen eds., Salinity Tolerance in Plants. Strategies for crop improvement. John Wiley and Sons, New York. 77—91.
7. Leopold, A.C. and R.P. Willing 1984. Evidence for toxicity effects of salt on membranes. In Staples, R.C. and G.H. Toenniessen eds., Salinity Tolerance in Plants. Strategies for crop improvement. John Wiley and Sons, New York. 67—76.
8. Maegawa, H., E. Usui, N. Uchida, T. Yasuda and T. Yamaguchi 1987. Studies on the mechanism of salt tolerance in rice (*Oryza sativa* L.). Relation between salt content and photosynthesis. Jpn. J. Trop. Agric. 31 : 92—98.

9. Naito, H., M. Tsuchiya and S. Kumano 1994. Physiological response to salinity in rice plant II. Relationship of sodium exclusion to transpiration and root-respiration rates in NaCl-treated rice plant. Jpn. J. Crop Sci. 63 : 320—325.
10. Nakagawa, T. 1989. Separation membrane. Fundamentals and utilization. Third edition. Sangyotosho, Inc., Tokyo. 43—92**.
11. Nakamura, A. 1993. Permeability of lipid membrane. In Nippon-kagakukai ed., The Membrane Is Living : Chemistry for common people No. 17. Dainippon-tosho, Inc., Japan. 53—72**.
12. Perumalla, C.J. and C.A. Peterson 1985. Deposition of Casparian bands and suberin lamellae in the exodermis and endodermis of young corn and onion roots. Can. J. Bot. 64 : 1873—1878.
13. Peterson, C.A. 1988. Exodermal Casparian bands : their significance for ion uptake by roots. Physiol. Plant. 72 : 204—208.
14. Tsuchiya, M., M. Miyake and H. Naito. 1994. Physiological Response to Salinity in Rice III. A possible mechanism for Na^+ exclusion by rice root under NaCl-stress conditions. Jpn. J. Crop Sci. 63 : 326—332.
15. ———, H. Naito, H. Ehara and T. Ogo 1992. Physiological response to salinity in rice plant. I. Relationship between Na^+ uptake and transpiration under different humidity and salinity conditions. Jpn. J. Crop Sci. 61 : 16—21*.
16. ———, ———, Y. Takagi and S. Kumano 1993. Salt exclusion rate in rice roots in relation to ion species. Sci. Rep. Fac. Agric. Okayama Univ. 82 : 17—23.
17. Wainwright, S.J. 1980. Plant in relation to salinity. Adv. Bot. Res. 8 : 221—261.
18. Yamanouchi, M. 1989. The mechanisms of salinity tolerance in rice plants. Relationships between the varietal difference of salinity tolerance and characteristics of absorption and translocation of sodium ion (2). Jpn. J. Soil Sci. Plant Nutr. 60 : 210—219*.

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