

Physiological Response to Salinity in Rice Plant

III. A possible mechanism for Na⁺ exclusion in rice root under NaCl-stress conditions*

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Abstract : The mechanism and varietal difference of Na⁺ exclusion in rice roots exposed to NaCl stress was studied in relation to reverse osmosis as the principle of ion exclusion. Exudates from the cut-surface of a root exposed to 20 mmol l⁻¹ NaCl solution with hydrostatic pressure of 294 and 686 kPa were sampled. Na⁺ concentration of the exudate from the whole cut-surface was at most 60% lower than that of the root medium. However, Na⁺ concentration of the exudate from the cut-surface of the stele was about 85% lower than that of the root medium. These results indicated that Na⁺ exclusion might occur at the exodermis and endodermis. The decrease of Na⁺ concentration of the exudate with hydrostatic pressure indicated that Na⁺ exclusion might be a non-metabolic process which could be driven by negative pressure induced by transpiration, and seemed to be based on reverse osmosis. From the results of X-ray micro-analysis, Na⁺ movement across the endodermis was less in IR28 than in Kala-Rata 1-24 (KR1). However, transpiration rate under saline conditions was higher in KR1, and it was considered to be supported by morphological characteristics of KR1 root, i.e. more roots per plant, longer and thicker roots, high ratio of stele area to the area of cross-section and more vessels per cross-section. It is concluded that the varietal difference in salt tolerance between KR1 and IR28 is related to the difference in the ability to maintain the negative pressure for ion exclusion by keeping the transpiration rate under saline conditions.

Key words : Endodermis, Exodermis, Na⁺ exclusion, Reverse osmosis, Rice, Stele, Salt tolerance.

塩分濃度に対するイネの生理反応に関する研究 第3報 高 NaCl 濃度下におけるイネの根の Na⁺ 排除機作について : 土屋幹夫・三宅 幸・内藤 整 (岡山大学農学部)

要 旨 : 高 NaCl 濃度下において認められるイネの根の Na⁺ 排除機能のメカニズムを、逆浸透を想定して検討するとともに、耐塩性品種 Kala-Rata 1-24 (KR1) と感受性品種 IR28 の差異を検討した。この想定からは、蒸散による負圧を駆動力としていることが推測されるため、溶液を根に加圧する装置を用い、一本の切断根を対象に NaCl 溶液を加圧して得られる出液の Na⁺ 濃度を測定した。その結果、切断面全体からの出液の Na⁺ 濃度は培地の Na⁺ 濃度に比べ最大でも 60% の低下であったのに対し、中心柱部分のみから得られた出液の Na⁺ 濃度は約 85% の低下を示した。これらの結果から、溶液が外皮および内皮を通過する際に Na⁺ の排除が行われていることが窺われた。また、溶液の物理的加圧によって排除機能が働いたことは、この機能が非代謝的な過程であることを示すと同時に、圧力を駆動力とした機能であり、逆浸透に酷似した機能であることが示唆された。X 線マイクロアナリシスの結果から、感受性品種である IR28 の根で、KR1 に比して、内皮を横切る Na⁺ の移動が、より起り難いことが明確になった。しかしながら、KR1 では、個体当たりの根数が多く、長く太い根が多いこと、横断面積に対する中心柱面積の割合が高く、横断面積当たりの後生大導管数も多いことが認められ、これらの形態的特徴が高 NaCl 濃度条件下における蒸散速度の維持に貢献しているものと考えられた。そして、この特性の違いが、Na⁺ 排除の駆動力の確保の差異を通じて、両品種の耐塩性程度の違いをもたらす理由の一つと考えられた。

キーワード : イネ、外皮、逆浸透、耐塩性、中心柱、内皮、Na⁺ 排除機能。

In our previous reports^{8,14)}, we showed that the Na⁺ exclusion efficiency of rice root expressed as transpiration stream concentration

factor of Na⁺ (TSCFNa⁺) under saline conditions changed with transpiration, and a higher transpiration rate led to higher exclusion efficiency, and consequently, resulted in low top-Na content. And exclusion efficiency at a certain transpiration rate was higher in IR28 (salt sensitive variety) than in Kala-Rata 1-24 (salt tolerant variety). In several reports, the contribution of active ion transport to salt

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exclusion^{4,5)}, or Na retention in the tissue of root and stem^{1,12,15,16)} was discussed. However, the mechanism of ion exclusion was not clear.

Various artificial membranes were used in various fields of industry. Among them, the ways of separating ions were restricted to electrodialysis and reverse osmosis. The electrodialysis membrane has permeability to either anion or cation depending on its quality, while rice root can exclude both Na⁺ and Cl⁻ ions at the same time. On the other hand, reverse osmosis, which is driven by pressure¹⁰⁾, has permeability mainly to water, and is used in desalting sea water⁹⁾ through the excluding capacity of salt, sugar and low molecular compounds like amino acid. Exclusion of Na⁺ and Cl⁻ by rice roots is similar to reverse osmosis in terms of exclusion efficiency, the necessity for a driving pressure and the excluding capacity of ions. It is well-known that plasma membrane has semipermeability. Hence, it is assumed that the mechanism of ion exclusion by rice root is basically reverse osmosis. In this study, the site and the mecha-

nism of ion exclusion in a rice root was investigated and the difference of salt tolerance between the above-mentioned varieties was discussed.

Materials and Methods

Two rice varieties, Kala-Rata 1-24 (KR1 ; salt tolerant) and IR28 (salt sensitive), were used. At the 12th leaf stage, rice plants grown in a glasshouse were treated with 0 and 100 mmol l⁻¹ NaCl solution for 12 hours at 30°C air temperature under 45% relative humidity conditions. After the treatment, the leaf-water potential of five leaf-positions on the main stem, which is considered to be the source of exclusion power, was measured using the pressure chamber method for 4 plants per plot. Results were presented as the average of 4 plants.

If ion exclusion in a rice root was driven by the pressure, Na⁺ must be excluded from the exudate coming through the vessel when a root is exposed to NaCl solution with hydrostatic pressure. To establish of this point, the

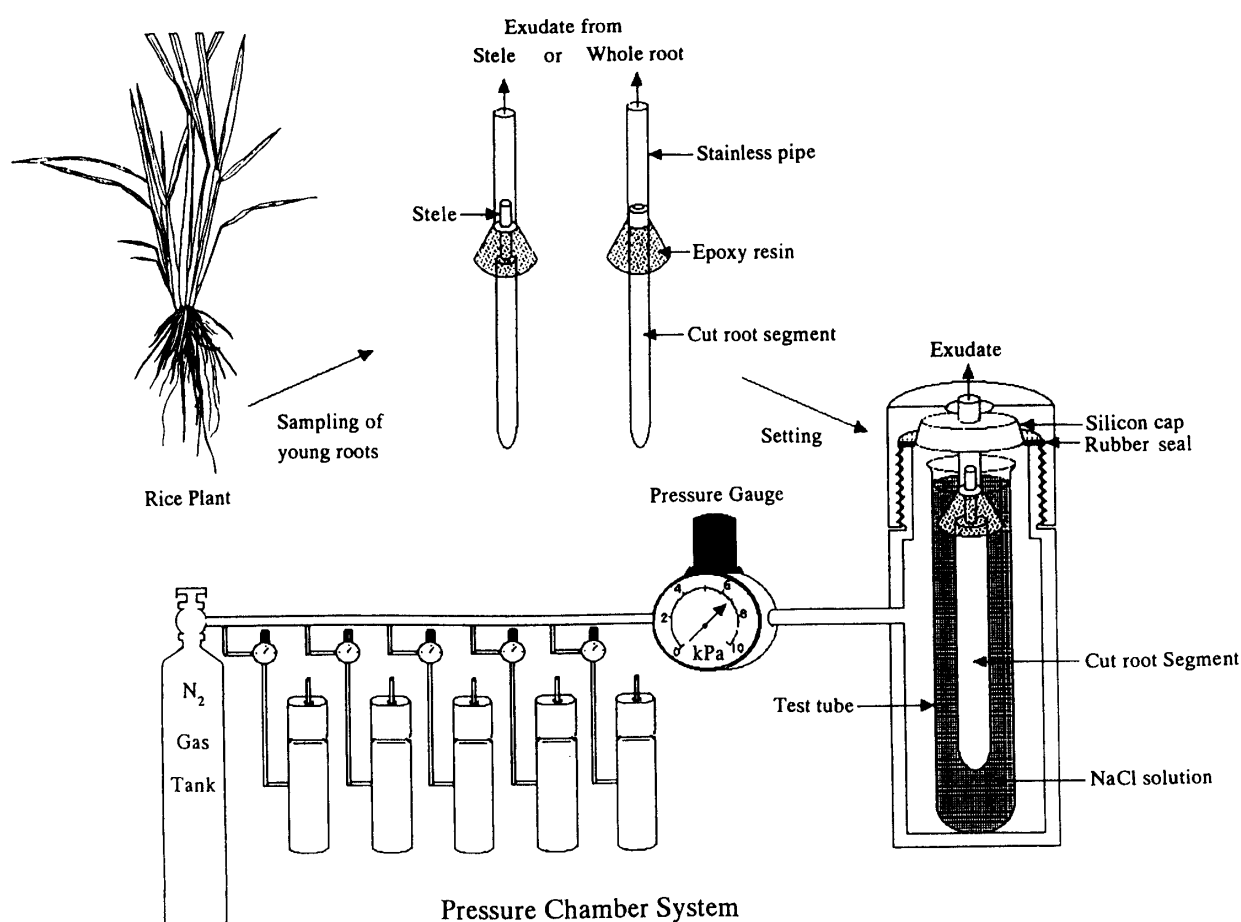


Fig. 1. Sampling procedure and the pressure chamber apparatus for studying the ion exclusion.

Na^+ concentration of the exudate from the root exposed to 20 mmol l^{-1} NaCl with hydrostatic pressure was measured. In order to gain exudate, a set of 6 self-made pressure chambers was used (Fig. 1). Sample roots were prepared from KR1 plants grown in solution culture at the 8th leaf stage. Excised roots were young and about 5 cm in length. A root was put in the stainless steel pipe, and glued with epoxy resin. A stainless steel pipe was fixed with a silicon rubber plug on the cap of a pressure chamber, and the cap was closed so that the root was submerged in a test tube with NaCl solution in the chamber. About $50 \mu\text{l}$ of the exudate from the root was gathered using a micro-syringe every 4 hours. Na^+ concentration in the exudate was measured by ion chromatography (Shimadzu HIC-6A). To measure the Na^+ concentration in the exudate from the stele, the root, whose lysigenous aerenchyma and epidermis were taken away, was also used. The operation to remove lysigenous aerenchyma and epidermis was done using tweezers and a scalpel under a stereoscopic microscope.

To clarify the varietal difference in the permeability of roots for Na^+ , Na^+ distribution in the cross-section of root treated with NaCl solution was investigated with X-ray micro-analysis. Rice seedlings at the 8th leaf stage were treated with 100 mmol l^{-1} NaCl solution for 10 minutes, 4 hours or 8 hours. After the treatment, the shoot was cut off at 2 cm from the shoot base. And to suppress the movement of water in the plant, the cut part was covered with silicon sealant. Roots of seedlings were rinsed with running water at 9 different rinsing durations (from 1 minute to 12 hours). At the end of each rinsing, 9 seedlings were

sampled for the measurement of Na content in root. Three plants for the X-ray micro-analysis by the scanning electron microscope (Hitachi S-800) were sampled after 1 minute, 4 and 12 hours of rinsing. For X-ray micro-analysis, to suppress the movement of Na^+ in the tissues, dried samples without solution fixation by FAA were used. Na concentration was analyzed at 5 sites in the outer and inner sites of the stele. Relative values of the outer Na concentration to the inner were calculated with the average.

To compare the morphological characteristics of root in two varieties in relation to transpiration, number of roots per plant, length and diameter of root, vessel number in the cross-section, the ratio of stele area to the total area of cross-section was investigated in about 5 seedlings at the 8th leaf stage. Root diameter was measured at 5 mm from the base by Laser Scan Diameter (Keyence: LS-3034, LS-3100). For the measurement of vessel number in the cross-section and ratio of the stele area to the total area of cross-section, a microscope with ocular micrometer was used.

Results and Discussion

Leaf-water potential of both varieties treated with 0 mmol l^{-1} NaCl solution was almost the same ($-0.4 \sim -0.5 \text{ MPa}$) in the whole leaves, but those treated with 100 mmol l^{-1} NaCl solution were lower and varied at the range of $-0.5 \sim 1.0 \text{ MPa}$ (Table 1). The pressure on xylem under saline conditions was not so different from the pressure which drove the industrial plant of reverse osmosis ($0.5 \sim 6.0 \text{ MPa}^{10)}$.

Figure 2 shows the change of Na^+ concentration in the exudate with an accumulated

Table 1. Effect of NaCl stress on leaf water potential in IR28 and Kala-Rata 1-24(-MPa).

Variety	NaCl (mmol l^{-1})	Leaf position					Mean
		n	n-1	n-2	n-3	n-4	
IR28	0	0.51	0.55	0.38	0.43	0.43	0.46
	100	0.90	1.02	0.50	0.76	1.00	0.84
KR1	0	0.42	0.53	0.58	0.40	0.53	0.49
	100	0.77	1.13	0.66	0.81	0.89	0.85

n leaf position = 12th leaf, Values are average of four plants.

Measured after 12hr NaCl treatment under 27°C , 45%RH, $460 \mu\text{Em}^{-2} \text{ s}^{-1}$ condition.

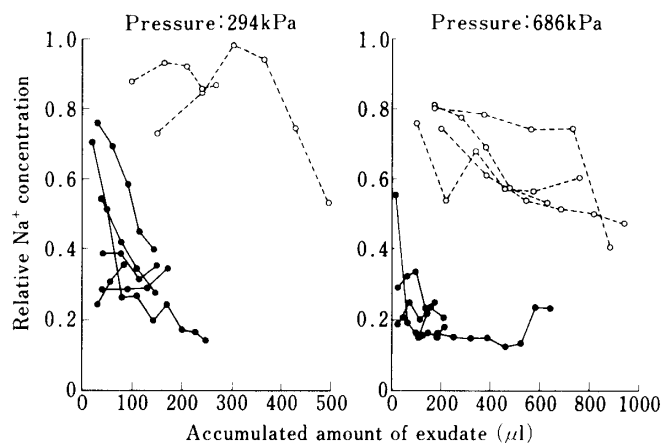


Fig. 2. Effect of applied pressure on Na⁺ concentration of the exudate of Kala-Rata 1-24 root exposed to 20 mmol l⁻¹ NaCl.

— : The exudate from the stele,
 : The exudate from the cross-section of a root.

amount of exudate. A line shows a result for a sample root. The Na⁺ concentration of the exudate from the cross-section of a root exposed to 20 mmol l⁻¹ NaCl solution at 294 and 686 kPa was at most 60% lower than that of the root medium (Fig. 2). On the other hand, Na⁺ concentration of the exudate from the stele was about 85% decreased, and Na⁺ exclusion efficiency was higher at 686 kPa than at 294 kPa. Excised roots used for this experiment were about 5 cm in length and 0.8 mm in diameter. Therefore, a sample root could contain 30 μl water at the maximum. If all this water was used for dilution of the exudate, the Na⁺ concentration of 60 μl exudate would be the half the Na⁺ concentration of treatment solution. Therefore, the fact that Na⁺ concentration of the exudate from stele was decreased to 20% of medium solution extended over 100 μl indicated the existence of Na⁺ exclusion by rice roots. The fact that Na⁺ was excluded by mechanical pressure also supported the hypothesis that the Na⁺ exclusion in rice roots was a non-metabolic process. This corresponds with the result investigated in the relationships of root respiration and transpiration to Na⁺ exclusion⁸⁾.

Munns and Passioura⁷⁾ reported that the osmotic pressure of leaf xylem sap, expressed from leaf tip by applying pressure to roots, decreased with increased transpiration, and they concluded that barley was able to exclude

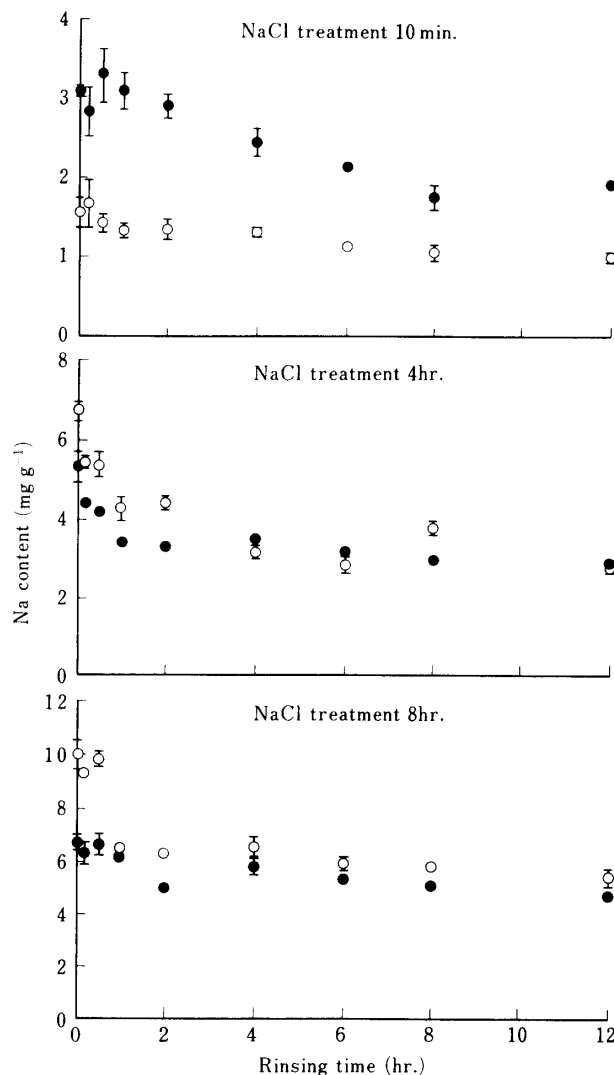


Fig. 3. Effect of rinsing duration on Na content of root.

○ : IR28 ● : Kala-Rata 1-24

Na⁺ and Cl⁻ from the transpiration stream. Furthermore, Munns⁶⁾ reported that Na⁺ concentration of xylem sap obtained by applying pressure to roots decreased with an increase of volume flux. However, the mechanism of exclusion and the site of exclusion were unknown. The separating efficiency of reverse osmosis is dependent on driving pressure, and the efficiency improves with higher driving pressure¹³⁾. Na⁺ concentration of exudate was lower at 686 kPa than at 294 kPa. This indicates that Na⁺ exclusion efficiency is also dependent on pressure. Na⁺ was excluded 10 to 60% in the exudate through the cut-surface of root, and 85% in the exudate through the stele of root. These results showed that this exclusion might occur at the exodermis and endodermis. Cereal crops have a hypodermis

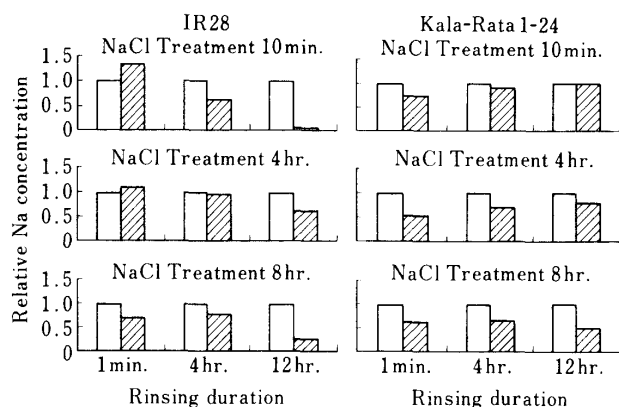


Fig. 4. Relative root Na concentration within and outside the stele under different NaCl and rinsing duration treatments.
□ : Within stele ▨ : Outside stele

which is the subepidermal cortical layers of roots^{2,3}). Exodermis is distinguished from hypodermis by its distinctive histologic characteristics. Exodermal cells have casparian bands¹¹). It is considered that casparian bands block the apoplastic movement of ions. Na^+ exclusion in rice roots may depend on the disturbance of ion flux by the casparian bands at the exodermis and endodermis.

If Na^+ was excluded mainly in crossing the endodermis, the Na^+ permeability of endodermis may cause the varietal difference of exclusion efficiency. On this basis, the Na^+ absorption and distribution in roots treated with 100 mmol l^{-1} NaCl was investigated. Na content of root at 1 minute rinsing in the 10 minute NaCl treatment was higher in KR1 than in IR28, but those in the 4 and 8 hour treatment were higher in IR28 (Fig. 3). And, Na content of roots decreased with rinsing duration in both varieties. These results indicated that Na content of roots in KR1 increased more easily than IR28 with the short-term NaCl treatment, but for the long-term NaCl treatment, Na content in IR28 increased more than that in KR1.

Radial Na^+ distribution in roots, which were rinsed with water for 1 minute, 4 and 12 hours, was investigated using X-ray micro-analysis. In 1 minute rinsing, Na content within the stele was higher than that outside of the stele in KR1, whose Na content was higher in the 10 minute-NaCl treatment (Fig. 4). However, in IR28, whose Na content was lower, Na content outside the stele was higher than that within the stele. In IR28, the ratio of

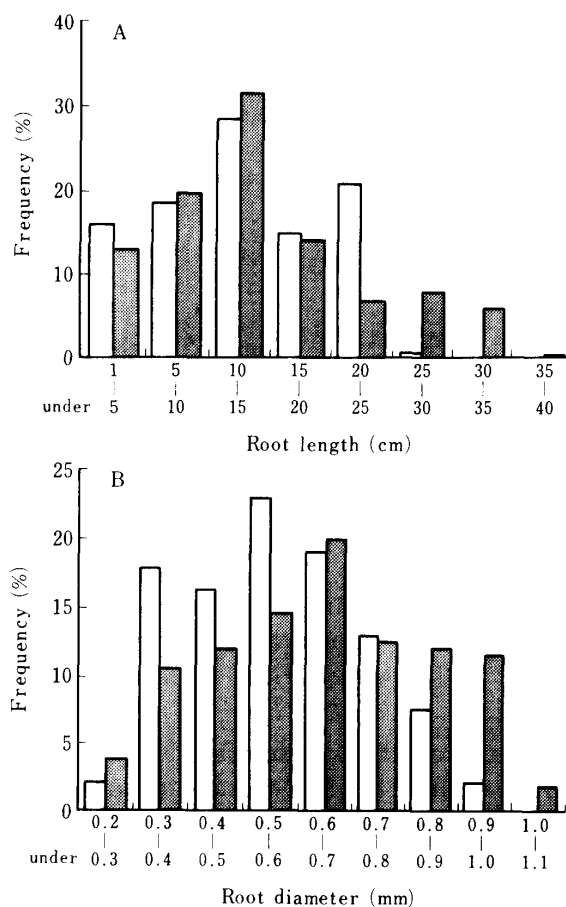


Fig. 5. Root length (A) and diameter (B) frequency distribution.
□ : IR28 (n=186) ▨ : Kala-Rata 1-24 (n=205)

Na content outside the stele to that within the stele was remarkably lowered with increasing of rinsing duration at any NaCl treatment duration. Therefore, it was considered that the decrease of Na content of root rinsed for 12 hours was caused by the reaching out of Na^+ from the cortex. However, in KR1, the ratio of Na content outside the stele to that within the stele did not change remarkably. The difference of Na content between the inside and the outside of the stele was extended in IR28 with rinsing duration, while it became smaller in KR1. These results indicated that Na^+ movement across the endodermis was more difficult in IR28 than in KR1. This result corresponded well with the varietal difference in transpiration stream concentration factor of Na^+ observed at the same transpiration rate¹⁴).

Morphological observation indicated that KR1 had more roots per plant and the roots were longer and thicker as compared with IR28 (Fig. 5). Moreover, the ratio of stele area

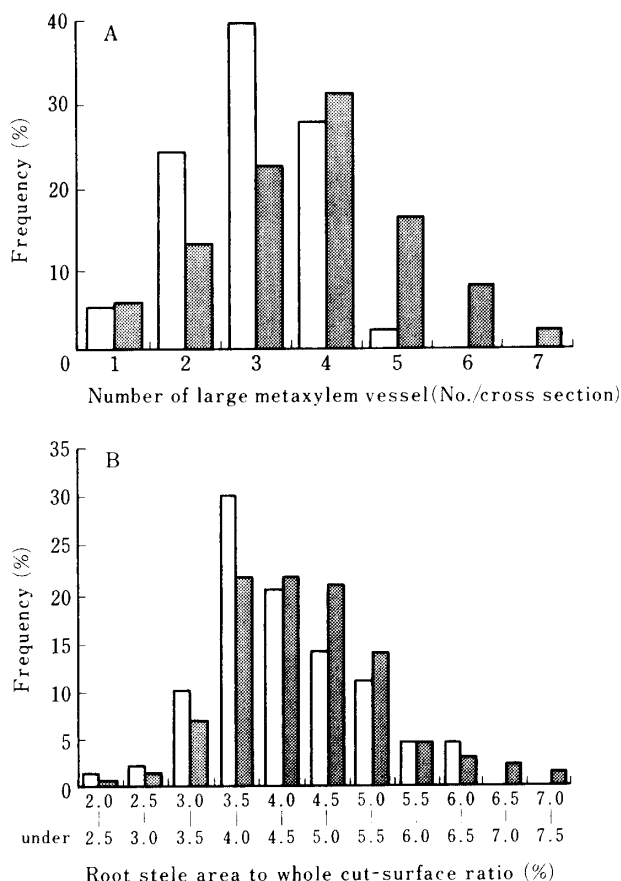


Fig. 6. Number of large metaxylem vessel (A), and root stele area to whole cut-surface ratio (B).
□ : IR28 (n=126) ■ : Kala-Rata 1-24 (n=128)

to the whole area of cut-surface was higher and vessel number per cross-section was also much more in KR1 (Fig. 6). It was considered that higher transpiration rate of KR1 under saline conditions compared with IR28¹⁴⁾ might be supported by these morphological characteristics of a root system.

Based on the above results, the varietal difference in salt tolerance between KR1 and IR28 could be understood as follows: under the same environmental conditions, especially with regard to the level of salinity and humidity, KR1 could exclude Na⁺ more efficiently than IR28, through the negative high pressure being produced by a high transpiration rate supported by the morphological characteristics of a whole plant, even though root quality of a membrane is poor in KR1 compared with IR28. And it is suggested that an improvement in root quality of a membrane and on plant characteristics for increasing the nega-

tive pressure in xylem produced by transpiration under saline conditions could contribute to the enhancement of salt tolerance in rice plants.

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** In Japanese with English summary.
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