Physiological Response to Salinity in Rice Plant

II. Relationship of sodium exclusion to transpiration and root-respiration rates in NaCl-treated rice plant*

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Abstract : In order to clarify the relationships of sodium exclusion to transpiration and root-respiration rates in 100 mmol l⁻¹ NaCl-treated rice plant, transpiration stream concentration factor of Na⁺ (TSCFNa⁺) was measured at different oxygen concentrations, pH and temperatures of root medium under different humidity conditions. It was confirmed that TSCFNa⁺ decreased hyperbolically with the increase of the transpiration rate, namely, sodium-exclusion efficiency increased with transpiration rate. However, TSCFNa⁺ and top-Na content were not clearly related to root-respiration. Neither was a difference found in the relationship between TSCFNa⁺ and transpiration rate measured at 20°C and 32°C, and at pH 4.0 and 7.0 of culture solution. These results imply that root respiration is not directly related to the sodium exclusion mechanism. It was shown that the sodium-exclusion mechanism also works for chlorine because of a positive correlation between TSCFNa⁺ and TSCFCl⁻. These results suggest that ion exclusion in rice plants may be based on a non-metabolic process and it is important to maintain a high transpiration rate for exclusion and growth under saline conditions.

Key words : Na content, Rice, Root-respiration rate, Sodium exclusion, Transpiration rate, Transpiration stream concentration factor.

塩分濃度に対するイネの生理反応に関する研究 第2報 Na⁺ 排除と蒸散および根部呼吸速度との関係：
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要 旨 : 蒸散流濃度係数によって表されるイネのナトリウム排除機能と蒸散および根部の呼吸との関係を明らかにするため、根部に通気する空気酸素濃度、培養液の温度および pH を変え、異なる温度条件下で 100 mmol l⁻¹ NaCl 濃度処理を行った。その結果、蒸散速度が高いほど蒸散流ナトリウム濃度係数 (TSCFNa⁺) は双曲線的に低下し、分離排除の効率が高まることが再確認された。しかしながら、根部の呼吸速度と TSCFNa⁺ および茎葉部 Na 含有率の間には明確な関係は認められず、根部の呼吸は Na の分離排除に直接関連しないものと考えられた。また、培養液の温度および pH を、20°C と 32°C、pH 4 と 7 に変えた場合でも、蒸散速度と TSCFNa⁺ の関係には差異は認められなかった。さらに、TSCFNa⁺ と蒸散流塩素濃度係数の間に正の直線関係が認められ、この排除機能が塩素イオンに対してもほぼ同様に働くことがわかった。これらの結果から、イネの根における Na⁺ および Cl⁻ の排除は非代謝的過程に基づくことが示唆され、また耐塩性の確保には、塩分条件下でも蒸散速度を高く維持できることが重要と推察された。

キーワード : イネ、根部呼吸速度、蒸散速度、蒸散流濃度係数、Na 含有率、ナトリウム排除機能。

The growth rate of rice plant under saline conditions is negatively correlated with top-Na content¹⁷⁾. Therefore, to maintain growth, it is important that sodium uptake and transport to the top are reduced. It was reported that active ion uptake is related to root metabolic activity¹⁵⁾, and passive ion uptake to transpiration rate³⁾. Therefore, both root respiration and transpiration may affect Na⁺ and Cl⁻ uptake under saline conditions. In our previous experiments¹³⁾, we showed that transpira-

tion affected Na⁺ uptake in rice under saline conditions. TSCFNa⁺ decreased with increase of transpiration rate, namely, sodium-exclusion efficiency increased with transpiration rate. However, the relationship between root respiration and sodium exclusion in rice plants is unknown. It is reported^{1,2,4)} that with root anoxia, the exclusion mechanism broke down so that a much greater amount of Na⁺ accumulated in the shoots. It is considered that this exclusion mechanism is related to active ion transport. Using metabolic inhibitors, Tanaka and Tadano¹¹⁾ showed that iron exclusion in rice roots was dependent on the metabolic activity of root.

In this study, the relationship of Na⁺ exclusion to transpiration and root-respiration rates

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was investigated.

Materials and Methods

Kala-Rata 1-24 (KR1; salt tolerant) and IR28 (salt sensitive) rice varieties were used. On April 14, 1992, germinated seeds were placed on a mesh in a plastic vessel filled with water. On April 25, 10 seedlings were transplanted at 3 cm intervals on a piece of Styrofoam placed in a 1/5000a Wagnerpot filled with the standard concentration of Kimura B nutrient solution. Culture solution was changed every week, and pH of culture solution was adjusted at 5.5 everyday. Seedlings at the 7th leaf stage were used for measurement. Three seedlings of each variety were set in the assimilation chamber. Transpiration rate and root-respiration rate were measured for 12 hours under the saline conditions of 100 mmol l^{-1} NaCl at 30.0°C of air temperature and 24.0°C of root medium (culture solution) under $1000 \mu\text{Em}^{-2}\text{s}^{-1}$ of photosynthetically active radiation, as in the previous report¹⁴. After the measurement, top-Na content was determined. Sodium in the dried and ground samples was extracted with boiling water for three hours, and then Na^+ concentration in the extract was determined by ion chromatography (Shimadzu HIC-6A). Transpiration stream concentration factor for Na^+ (TSCFNa^+) was calculated as follows:

$$\text{TSCFNa}^+ = [(\text{top Na content after measurement} - \text{top Na content before measurement}) / \text{the amount of transpiration}] / \text{Na concentration in root medium}$$

Transpiration stream concentration factor for Cl^- (TSCFCl^-) was also computed in the same way as mentioned above. TSCFNa^+ was measured at six levels of oxygen concentration in the root medium, namely, 0, 0.5, 1, 5, 10, 21% (Experiment 1). Oxygen concentration was adjusted by N_2 , CO_2 and O_2 gas mixing.

In the second experiment, TSCFNa^+ was measured at 20.0 and 32.0°C of culture solution under 21% oxygen concentration. The measurement was made ten times at different air humidity conditions from August 17 to September 8, 1992.

In the third experiment, TSCFNa^+ was measured at two different pH levels, i. e. pH4.0 and 7.0, as pH affects the electrochemical gradient in the kinetics of ion transport. The culture solution was maintained at

Fig. 1. Root-respiration rate at different oxygen concentrations of root medium.

● : KR1, ○ : IR28.

24.0°C under 21% oxygen concentration. In experiment 1 and 2, TSCFCl^- was not measured, because chloride is absorbed in parallel with sodium absorption^{12,16}. However, in this experiment, TSCFCl^- was also measured because pH may have a different effect on TSCFNa^+ and TSCFCl^- . Chloride was extracted according to the procedure in the Methods of Crop Physiological Experiment⁵, and Cl^- concentration in the extract was also determined by ion chromatography.

Results and Discussion

Figure 1 shows root-respiration rate at different oxygen concentration of root medium. The respiration rate was not remarkably different in the range of more than 5% oxygen concentration. However, it seemed to be suppressed below 1% oxygen concentration, as far as root-respiration rate was measured in CO_2 evolution. TSCFNa^+ measured at six oxygen levels hyperbolically decreased with the increase of the transpiration rate (Fig. 2). This confirmed our previous result¹³ and corresponded with the result reported by Munns⁸). He found that sodium concentration in xylem sap flowing to shoots of NaCl-treated barley decreased with the increasing volume flux transpiration stream. However, relationships were not found between the root-respiration rate and TSCFNa^+ (Fig. 3), and between the root-respiration rate and top-Na content (Fig. 4). From these results, it was concluded that root respiration was not direct-

Fig. 2. Relationship between transpiration rate and transpiration stream concentration factor of Na⁺ (TSCFNa⁺) measured under different oxygen concentrations of root medium.

●—: KR1, ○···: IR28.

Fig. 3. Relationship between root-respiration rate and TSCFNa⁺ measured under different oxygen concentrations of root medium.

●: KR1, ○: IR28.

Fig. 4. Relationship between root-respiration rate and top-Na content measured under different oxygen concentrations of root medium.

●: KR1, ○: IR28.

Fig. 5. Relationship between transpiration rate and TSCFNa⁺ measured at different culture-solution temperatures.

●—: KR1 32°C, ○—: IR28 32°C,
■—: KR1 20°C, □—: IR28 20°C.

ly related to sodium exclusion under saline conditions.

TSCFNa⁺ measured in relation to the temperature of culture solution also hyperbolically decreased with increase of transpiration rate (Fig. 5). Transpiration rate at 32°C was higher than at 20°C, and as a result, TSCFNa⁺ at 32°C was lower than at 20°C. However, the relationship between transpiration rate and TSCFNa⁺ seemed to be in the same at different temperatures. This was examined¹⁰⁾ after

converting a hyperbolic relation into a linear one by computing the logarithm of the transpiration and TSCFNa⁺. The result showed that the relationship between transpiration rate and TSCFNa⁺ was not significantly changed by the temperature of the culture solution (Fig. 6).

On the other hand, it was reported⁷⁾ that viscosity of solution changes with solution temperature, becoming viscous at a lower temperature. The water viscosity at 20°C is

Fig. 6. Relationship between transpiration rate and TSCFNa⁺ measured at different culture-solution temperatures.

Fig. 7. Relationship between root-respiration rate and top-Na content measured at different culture-solution temperatures.

●: KR1 32°C, ○: IR28 32°C,
■: KR1 20°C, □: IR28 20°C.

Table 1. Result of multiple regression analysis relating TSCFNa⁺ (X1), transpiration rate (X2) and root-respiration rate (X3) to top-Na content (Y).

var.	Multiple correlation	Partial regression coefficient (β)	Standard β	Partial correlation
KR1	0.964**	—	—	—
(X1)	—	30.4701	0.805	0.851**
(X2)	—	-0.0614	-0.173	-0.263ns
(X3)	—	-0.0007	-0.010	-0.024ns

** and ns indicate a significance at 1% level and non-significance, respectively.

31% higher than at 32°C. So, it was considered that one reason why transpiration was lower at 20°C was the increase in viscosity. This implies that the difference in solution temperature did not affect ion exclusion efficiency directly, in short, being through the decrease in transpiration. Figure 7 showed that the root-respiration rate was lower at 20°C than at 32°C and top-Na content seemed to increase with the decrease of root-respiration rate in KR1, but not in IR28. However, even in KR1, based on the result of the multiple regression analysis on the relationships of TSCFNa⁺, transpiration rate and root-respiration rate to top-Na content, it was found that root-respiration rate did not affect top-Na content significantly (Table 1).

Both TSCFNa⁺ and TSCFCl⁻ measured at different pH levels decreased hyperbolically with the increase in transpiration rate (Fig. 8, 9). Using logarithms of TSCFNa⁺ and transpi-

ration rate, the difference in the regression equations at two pH levels was also examined. The result showed that the regression equations were not significantly different between pH4 and pH7. Therefore, it was considered that exclusion of both ions in rice roots might not be directly related to an ion exchange mechanism such as H⁺ pump. TSCFNa⁺ was highly correlated with TSCFCl⁻ (Fig. 10), and the ratio of exclusion efficiency for Na⁺ to that for Cl⁻ was almost constant. Therefore, it was suggested that the exclusion mechanism works not only for Na⁺ but also for Cl⁻.

From these results, it was strongly supposed that ion exclusion in rice roots was a non-metabolic process and not directly related to the metabolism of root respiration. Tanaka and Tadano¹²⁾ found that metabolic inhibitors broke down iron exclusion, and they concluded that the exclusion was supported by a metabolic process. However, we could see it in

Fig. 8. Relationship between transpiration rate and TSCFNa⁺ measured at different pH levels.

●— : KR1 pH4, ○— : IR28 pH4,
■— : KR1 pH7, □— : IR28 pH7.

Fig. 9. Relationship between transpiration rate and transpiration stream concentration factor of Cl⁻ (TSCFCl⁻) measured at different pH levels.

●— : KR1 pH4, ○— : IR28 pH4,
■— : KR1 pH7, □— : IR28 pH7.

their data that metabolic inhibitors also decreased water uptake and there was a positive correlation between iron exclusion and water uptake. Therefore, some metabolic inhibitors may affect ion exclusion through decreasing transpiration.

Using all the data of the three experiments, it was found that the amount of Na⁺ translocated to the top decreased with the increase of transpiration rate in both varieties (Fig. 11). This suggested that the increase of tran-

Fig. 10. Relationship between TSCFNa⁺ and TSCFCl⁻ measured at different pH levels.
●— : KR1, ○— : IR28.

Fig. 11. The effect of transpiration rate on the amount of Na translocated to top and TSCFNa⁺.

●— : KR1, ○— : IR28.

Symbols with 0 are values measured at 0% oxygen concentration of root medium.

spiration contributed to the decrease in the amount of Na⁺ translocated to the top through the lowering of TSCFNa⁺. However, the amount of Na⁺ translocated to the top increased at 0% oxygen concentration. It was reported that ethanol and lactic acid accumulated in the roots under anoxia⁹⁾, and that ethanol injured the protoplasm membrane of roots⁶⁾ because of its fat solubility. The increased permeability by the accumulated ethanol at 0% oxygen concentration may cause the decrease of Na⁺ exclusion. Though the varietal difference in the exclusion efficiency was not so pronounced, IR28 had a

tendency to exclude ions more efficiently than KR1 at a same transpiration rate, as the shown results of our previous paper¹⁴⁾. These results may indicate the change or difference in quality of a root as an ideal membrane. However, it still remains unclear why the amount of Na^+ translocated to the top increased at 0% oxygen concentration. Investigation of varietal difference in the exclusion and internal structure of root may give us a possible breeding objective for salt tolerance. In any case, the oxygen concentration at more than 1% did not affect the Na^+ exclusion, and even at 0% oxygen concentration, TSCF Na^+ increased by 5% only. This result also supports the hypothesis that the exclusion in rice is a non-metabolic process. It has been considered that the growth of rice plants declined because of the accumulation of Na^+ accompanied by transpiration. However, Munns⁸⁾ showed that Na^+ concentration in xylem sap from a plant decreased with the increase of transpiration rate, and that Na^+ flux was almost constant. He mentioned that the cause of reduced growth appeared to be a change within the roots, associated with their ionic or water status. On the other hand, in this study, it was shown that Na^+ concentration of absorbed solution became lower with the increase of transpiration and consequently, the amount of Na^+ translocated to the top was decreased. This result indicates that decline of rice growth under saline conditions is caused and accelerated through lowering exclusion efficiency by the decrease of transpiration, rather than decreasing the amount of Na^+ translocated to the top. Therefore, it may be important to maintain a high transpiration rate for rice plants under saline conditions.

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