

## Salt Tolerance of Cultivated Rice Varieties from Vietnam

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### Abstract

This study was conducted to analyse the salt tolerance of rice varieties from Vietnam at the germination and seedling stages. The effects of different kinds of salts upon seedling growth were also examined. Germination rate in 1.5% NaCl solution (24.4 dS m<sup>-1</sup>) ranged from 3% (Mot Bui) to 81% (OM1630-50). The salt tolerance at the germination and seedling stages was not positively correlated with each other. Variability in salt tolerance was obvious between tall and semi-dwarf groups and within each group. Although no variety survived at 16 dS m<sup>-1</sup>, Thang Nong Do, a tall variety, was found to display a high salt tolerance. OM997, a moderately salt-tolerant semi-dwarf variety, would be a good example of breeding efforts in combining salt tolerance and high yield. The effect of salinity on shoot growth was less severe for older seedlings. A significant difference was observed in the effects of different kinds of salts, among which calcium chloride exhibited the severest effect and sodium sulfate was least harmful.

**Additional key words:** *Oryza sativa* L., seed germination, seedling growth, varietal screening

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## Introduction

Rice is a staple food for millions of people in the world. To sustain the food supply for an increasing population of rice eaters, the world rice production must be increased further. Unfortunately, several biotic and abiotic factors lower rice production. Among them, soil salinity is one of the most wide-spread soil problems<sup>9, 19</sup>. In Vietnam, salinity is a major abiotic constraint lowering rice production. Particularly in the Mekong Delta, out of 1.8 million ha of rice land, 500,000 ha are salt-affected. Due to the intensive cultivation of rice at present, i.e., increase in number of rice croppings to 2-3 per year, have lead to the more intrusion of salt water from the sea, because of limited fresh water resources. Therefore, breeding of rice varieties tolerant to salinity is essential to achieve sustainable rice production in the region.

Rice is a relatively salt-sensitive crop and its threshold level of salinity was estimated to be an electrical conductivity of around  $3 \text{ dS m}^{-1}$ , as compared to  $6 \text{ dS m}^{-1}$  for wheat and  $8 \text{ dS m}^{-1}$  for barley<sup>14</sup>. However, the degree of salt tolerance in rice varies both with the plant growth stages and the types of salts used. Mass-screening of varieties for salt tolerance has been normally carried out at the seedling stage and under NaCl stress<sup>3</sup>. Recently, Aslam and Qureshi<sup>6</sup> and Ahmed and Gupta<sup>2</sup> have introduced a screening method for salinity tolerance at the germination stage, and using this method, Tobita *et al.*<sup>18</sup> analysed the geographical distribution of salt tolerance in rice.

It is generally recognized that local varieties of the traditional, non-dwarf plant type are more tolerant to salinity than short varieties harboring dwarfing genes<sup>21</sup>. Yeo *et al.*<sup>21</sup> speculated that the frequency of the gene(s) conferring salinity tolerance had decreased during the selection of agronomically favorable semi-dwarf varieties by farmers and breeders.

This study aimed at investigating the effects of salinity upon germination and seedling growth in tall and semi-dwarf rice varieties from Vietnam; and

also to evaluate the effects of different kinds of salts applied to rice seedlings at different ages.

## Materials and Methods

### 1) Salt tolerance of rice varieties at germination and seedling stages

Seven tall varieties and eight semi-dwarf varieties were used. Except for Pokkali, a variety from Sri Lanka, all the tall varieties, Trang Tep, Trang Lun, Mot Bui and Than Nong Do originated from Vietnam. Among eight semi-dwarf varieties, four were bred at the International Rice Research Institute: IR28, IR64, OM90-9 (IR35546-17-3-1-3) and MTL110 (IR54752-23-19-16-10-3) and four were developed at the Cuu Long Delta Rice Research Institute, Vietnam: OM1327, OM269, OM997 and OM1630-50.

To test the salt tolerance at the germination stage, 30 seeds of each variety were placed in a 10-cm petri dish containing 10 ml of 1.5% (w/v) NaCl solution or distilled water as control<sup>18</sup>. The seeds were incubated at 30°C under a photoperiod of 16/8-hours (day/night). The germination rate was determined after 10 days based upon the emergence of the radicle and calculated as follows:

$$\text{Germination rate (\%)} \text{ under salt stress} \\ = \left[ \frac{\text{(germination rate in 1.5\% NaCl solution)}}{\text{(germination rate in control)}} \right] \times 100.$$

To test the salt tolerance at the seedling stage, 5-day-old seedlings were placed in holes arranged in a 10-mm thick styrofoam board with a nylon net bottom, which was floated on a Yoshida<sup>22</sup>'s nutrient solution containing  $\text{NH}_4\text{NO}_3$  ( $114.3 \text{ mg L}^{-1}$ ),  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (50.4),  $\text{K}_2\text{SO}_4$  (89.3),  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (110.8),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (405),  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  (1.9),  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  (0.1),  $\text{H}_3\text{BO}_3$  (1.2),  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (0.1),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0.1),  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (9.7) and citric acid (14.9). When the seedlings were 9-day old, they were transferred to nutrient solutions containing different levels of salt, which were prepared by dissolving NaCl to reach an electrical conductivity of 4, 8, 12 or  $16 \text{ dS m}^{-1}$ . A

completely randomized design was adopted to accommodate 15 varieties  $\times$  5 treatments (including control) with three replications. The materials were maintained in a glass house at 28°C under natural light. Solution was replaced by a fresh one at 5-day intervals and pH was maintained at 5.2.

Observations were taken at the start of salinization and 14 days after the treatment for the following characters: shoot length, total dry weight (shoot + root), ratio of shoot to root dry weight. Survival rate was also determined by recording the numbers of dead and alive seedlings subjected to the salinity treatments for 14 days, in which the varieties were classified into three groups: R where 81-100% of the plants survived, M where 51-80% survived, and S where 0-50% survived.

## 2) Effect of different salts applied at different seedling ages on seedling growth

Four varieties were used including two tolerant varieties (Pokkali and Than Nong Do), identified in the first part of this study, and two susceptible varieties (IR29 and Hitomebore). Salinity (12 dS m<sup>-1</sup>) was induced by adding separately four kinds of salts: NaCl, CaCl<sub>2</sub>, Na<sub>2</sub>SO<sub>4</sub> and a mixture of them (NaCl:CaCl<sub>2</sub>:Na<sub>2</sub>SO<sub>4</sub>=1:1:1). The method for growing seedlings in solution was described earlier. Salinity treatment was started at different seedling ages: 6-, 9-, 12- or 15-day-old. A completely randomized design was adopted to accommodate 4 varieties  $\times$  4 ages  $\times$  5 treatments (including control) with two replications. Shoot length at the start of salinization and at 14 days after the treatment was recorded.

## Results

### 1) Germination rate of rice varieties under salt stress

The varieties showed a wide range of germination rates in 1.5% NaCl solution (Table 1). Among the tall varieties, Pokkali showed the highest germination rate (85%), while Mot Bui, the lowest (3%). Germination rate of other tall varieties

Table 1. Germination rate of tall and semi-dwarf rice varieties under salt stress (1.5%NaCl)

Variety	Germination rate (%)
<b>Tall variety</b>	
Nang Huong Ran	50b <sup>†</sup>
Nang Thom C D	20de
Trang Tep	10de
Trang Lun	15de
Mot Bui	3e
Than Nong Do	25cd
Pokkali	85a
<b>Semi-dwarf variety</b>	
OM1327	49b
OM269	41b
OM90-9	70a
OM997	77a
OM1630-50	81a
MTL110	18de
IR64	43b
IR28	4e

<sup>†</sup>Means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

varied between 10-50%. In the semi-dwarf variety group, OM1630-50, OM269 and OM997 displayed a high germination rate (70-81%) while MTL110 (18%) and IR28 (4%), the lowest.

### 2) Tolerance of rice seedlings to different salt levels

Shoot growth of 15 varieties was evaluated at 4 levels of salt stress (4, 8, 12 and 16 dS m<sup>-1</sup>) plus control (without salt). Among the tall varieties, shoot growth of Trang Lun and Trang Tep was decreased by 4 dS m<sup>-1</sup> salinity (Fig. 1A). At 8 dS m<sup>-1</sup>, shoot growth of Nang Thom C D and Mot Bui was significantly suppressed. At a salinity level of 12 dS m<sup>-1</sup>, Pokkali and Than Nong Do retained more than 65% of their relative shoot growth compared with the control.

In the semi-dwarf variety group, shoot growth of all the varieties except for OM1327 and OM997 severely decreased when the salinity level increased (Fig. 1B). At 8 dS m<sup>-1</sup>, OM1327 showed a higher relative increase of shoot length than the other varieties, while OM997 performed better at 12 dS m<sup>-1</sup>.

In addition to shoot elongation, rice varieties also differed from each other in dry weight growth under salt stress (Figs. 2A and 2B). In the tall variety group (Fig. 2A), Than Nong Do, Nang Thom C D and Pokkali showed a higher relative increase in dry weight as compared to the other varieties at 12 dS m<sup>-1</sup>. The relative increase in Pokkali was 83% of the control at 16 dS m<sup>-1</sup>, while in the other varieties, below 75%. Semi-dwarf varieties showed a similar dry weight reduction when the salinity level increased (Fig. 2B). The relative dry weight of OM997, OM1630-50 and IR64 was, however, higher than that of the other varieties at 12 dS m<sup>-1</sup>.

The ratio of shoot to root dry weight (T/R

ratio) ranged between 1.6-4.0 among the varieties (Table 2). The T/R ratio was reduced by salinity but the decrease was significant only in OM90-9 and OM1630-50 at high salt levels.

Table 3 shows the survival rate of tall and semi-dwarf varieties after 14 days of exposure to salinity stress. When the salinity level increased to 4 dS m<sup>-1</sup>, plant survival of OM269 and MTL110 decreased. The survival rate of most of the varieties declined at 8 dS m<sup>-1</sup>, and only 4 varieties, Pokkali, Than Nong Do, Nang Huong Ran and OM997 showed a high survival rate at this salt level. Pokkali and Than Nong Do still survived at 12 dS m<sup>-1</sup> but the rate decreased to less than 50% at 16 dS m<sup>-1</sup>.

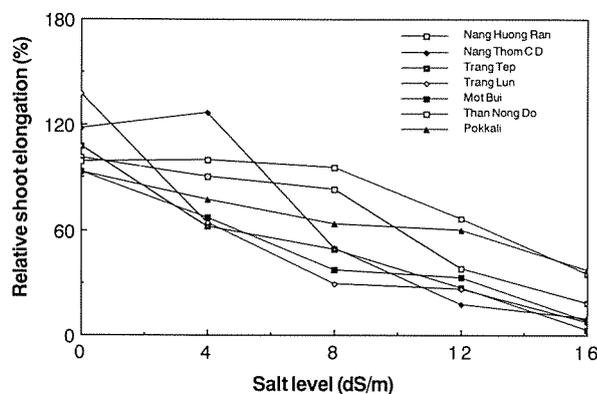


Fig. 1A

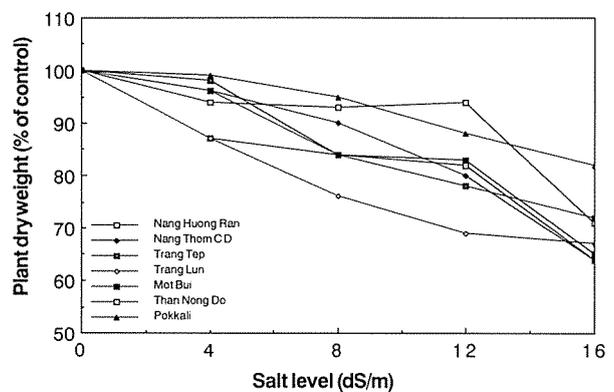


Fig. 2A

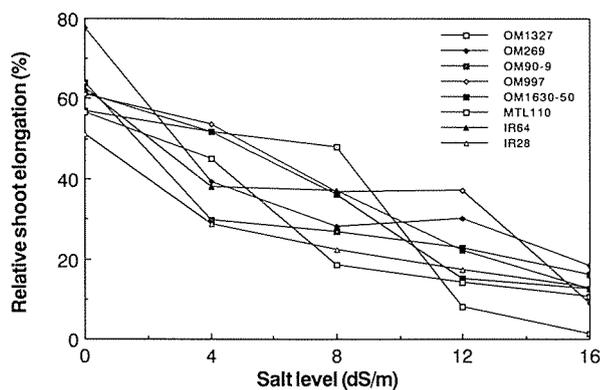


Fig. 1B

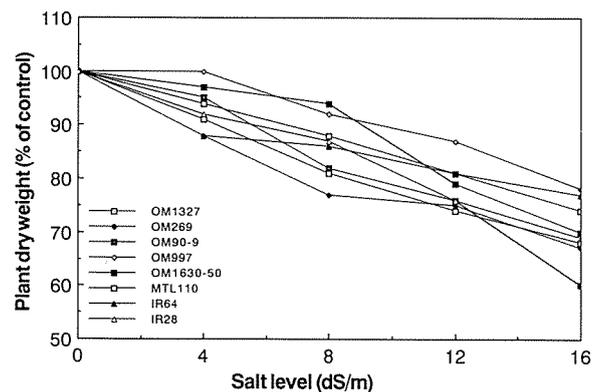


Fig. 2B

Fig. 1. Relative shoot elongation (%) of tall varieties (A) and semi-dwarf varieties (B) during the 14 day period of salt treatments. L.S.D. at the 5% level was 16.

Fig. 2. Relative increase in plant dry weight to control in tall varieties (A) and semi-dwarf varieties (B) under different levels of salinity for 14 days. L.S.D. at the 5% level was 8.3.

Table 2. Ratio of shoot to root dry weight of tall and semi-dwarf rice varieties under different salt levels

Variety	Salinity level (dSm <sup>-1</sup> )				
	control	4	8	12	16
<b>Tall variety</b>					
Nang Huong Ran	2.8 <sup>†</sup>	3.3	3.3	2.5	2.4
Nang Thom C D	3.2	2.6	2.1	2.5	2.9
Trang Tep	3.9	4.0	3.2	3.2	3.0
Trang Lun	2.8	3.1	2.7	3.4	2.7
Mot Bui	3.2	3.0	3.2	3.7	2.9
Than Nong Do	3.7	3.5	4.0	3.4	3.0
Pokkali	3.2	3.1	3.5	3.3	2.9
<b>Semi-dwarf variety</b>					
OM1327	2.8 <sup>†</sup>	3.3	3.3	2.5	2.4
OM269	2.8	2.1	3.0	2.4	2.3
ON90-9	3.4	2.6	2.9	2.3	1.8
OM997	3.2	3.2	2.7	2.9	2.7
OM1630-50	3.2	2.8	2.6	2.5	1.8
MTL110	2.4	3.0	2.6	2.4	1.6
IR64	2.6	2.4	3.1	2.3	2.1
IR28	2.6	3.0	2.8	3.3	2.8

<sup>†</sup>LSD 5%=0.9

Table 3. Plant survival rating of tall and semi-dwarf rice varieties after the 14 day period of salt treatments

Variety	Salinity level (dSm <sup>-1</sup> )				
	control	4	8	12	16
<b>Tall variety</b>					
Nang Huong Ran	R <sup>†</sup>	R	M <sup>‡</sup>	S <sup>§</sup>	S
Nang Thom C D	R	R	S	S	S
Trang Tep	R	R	S	S	S
Trang Lun	R	R	S	S	S
Mot Bui	R	R	S	S	S
Than Nong Do	R	R	R	R	S
Pokkali	R	R	R	R	S
<b>Semi-dwarf variety</b>					
OM1327	R	R	M	S	S
OM269	R	M	S	S	S
OM90-9	R	R	S	S	S
OM997	R	R	R	S	S
OM1630-50	R	R	S	S	S
MTL110	R	M	S	S	S
IR64	R	R	S	S	S
IR28	R	R	S	S	S

<sup>†</sup>R: 81-100% of plants survived

<sup>‡</sup>M: 51-80% of plants survived

<sup>§</sup>S: 0-50% of plants survived

### 3) Effects of different salts applied at different seedling ages on seedling growth

Table 4 shows the relative increase in shoot length during the 14 day period of salt treatments. The effects of salinity were influenced by the growth stage at which the seedlings were exposed to salts. When the seedling age increased from 6- to 15-day-old, the increase of the shoot length under NaCl stress rose from 31.5% to 75.2% in Pokkali, from 33.8% to 67.2% in Than Nong Do, from 2.7% to 34.9% in IR29 and from 10.6% to 53.0% in Hitomebore. A similar trend was also observed when the seedlings were exposed to other salts and their mixture. Over four salt kinds, the effects of salinity were severer on younger seedlings than

Table 4. Effects of seedling age (D) and salt kind (S) on the shoot elongation (%) of tall and semi-dwarf rice varieties (V) during the 14 day period of salt stress (EC=12 dS m<sup>-1</sup>)

Salt kind (S)	Variety (V)				
	Seedling age (D)	Pokkali	Than Nong Do	IR29	Hitomebore
Control					
6-day	100.7b <sup>†‡</sup>	93.6a	76.0a	105.0a	
9-day	125.4a	94.1a	77.2a	107.7a	
12-day	96.9b	100.3a	73.2a	90.3a	
15-day	87.2b	106.4a	63.2a	98.4a	
NaCl					
6-day	31.5c	33.8b	2.7b	10.6b	
9-day	50.1bc	43.7b	3.0b	15.4b	
12-day	64.3ab	51.8ab	12.5b	17.5b	
15-day	75.2a	67.2a	34.9a	53.0a	
CaCl <sub>2</sub>					
6-day	16.0c	23.5b	8.1a	6.7b	
9-day	27.8bc	35.9b	9.3a	8.1ab	
12-day	40.5b	42.7a	16.7a	15.1a	
15-day	62.0a	70.4a	17.4a	28.6a	
Na <sub>2</sub> SO <sub>4</sub>					
6-day	35.9c	33.0c	12.8b	12.1c	
9-day	69.7b	55.7b	27.7ab	25.9bc	
12-day	74.4b	52.2bc	24.7ab	36.3b	
15-day	96.4a	100.1a	43.4a	56.4a	
Mixture (1:1:1)					
6-day	33.0c	35.7b	5.2a	7.8b	
9-day	53.4b	51.6b	12.9a	20.3b	
12-day	68.8ab	49.0b	14.0a	25.4b	
15-day	77.7a	80.6a	24.1a	54.7a	

<sup>†</sup>In a column under each salt, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test

<sup>‡</sup>LSD 5% for 2 × V × S × D=19.9

on older ones.

A significant difference was observed among the effects of different kinds of salts on shoot growth of rice varieties (Table 4). In Pokkali, the increase of shoot length 14 days after exposure to salinity was 31.5% under sodium-chloride salinity, 16.0% under calcium chloride, 35.9% under sodium sulfate and 33.0% under the salt mixture, when the salts were applied to 6-day-old seedlings. In IR29 seedlings of the same age, shoot elongation was 2.7% under NaCl, 8.1% under CaCl<sub>2</sub>, 12.8% under Na<sub>2</sub>SO<sub>4</sub> and 5.2% under the mixture. When 15-day-old seedlings of IR29 were exposed to salts, the increase of shoot length was 34.9% under NaCl, 17.4% under CaCl<sub>2</sub>, 43.4% under Na<sub>2</sub>SO<sub>4</sub> and 21.4% under the salt mixture. Over four seedling ages and over four varieties, it was observed that CaCl<sub>2</sub> retarded shoot growth more than the other salts while Na<sub>2</sub>SO<sub>4</sub> was less detrimental, particularly in tolerant varieties.

## Discussion

Although rice is classified as a relatively salt-sensitive crop<sup>7)</sup>, there is a wide range of variation in salt tolerance among the varieties. Previous studies showed that seed germination rate in 1.5% NaCl solution (equivalent to 24.4 dS m<sup>-1</sup>) is one of the good indicators of salt tolerance, in which the rates of more than 350 rice varieties ranged from 0% to 100%<sup>18)</sup>. In this study, among seven tall varieties tested, Pokkali exhibited the highest germination rate (85%) under salinity. Under the same conditions, three out of eight semi-dwarf varieties showed relatively high germination rates (70-81%).

The survival scoring of plants based on visible symptoms is also a reliable and simple indicator of salinity tolerance and frequently used as a screening criterion<sup>15, 21)</sup>. In our studies, salt-sensitive varieties showed a seedling mortality starting at a salt level as low as 4 dS m<sup>-1</sup>. The survival of most varieties significantly decreased at a salinity level of 8 dS m<sup>-1</sup>. High survival rate (>81%) at 12 dS m<sup>-1</sup> was observed in only two

varieties, Pokkali and Than Nong Do. The seedlings of these varieties, however, died at 16 dS m<sup>-1</sup>. Based on the survival rating, out of fifteen varieties, Pokkali and Than Nong Do were salt-tolerant, and Nang Huong Ran and OM997 were moderately salt-tolerant.

Shoot growth of salt-sensitive varieties decreased significantly at a salt level as low as 4 dS m<sup>-1</sup>. When the salinity level was increased to 8 dS m<sup>-1</sup>, shoot growth of most of the varieties was severely affected. Three varieties (Pokkali, Than Nong Do, OM997), however, were able to maintain high shoot growth rates under the salt stress. The relative increase of shoot length in Pokkali and Than Nong Do was 60% at 12 dS m<sup>-1</sup>, while it was below 40% in the other varieties. Therefore, shoot elongation rate can also be considered as a criterion to evaluate salt tolerance in rice.

Salinity exerted a less detrimental effect on dry weight growth than on shoot elongation. At 12 dS m<sup>-1</sup>, in most of the varieties plant dry weight value was greater than 70% of the control plants. Pokkali showed the highest relative dry weight at 16 dS m<sup>-1</sup>. The ratio of shoot to root dry weight (T/R ratio) in most varieties did not change significantly when the salinity level increased, indicating that the decrease in shoot and root dry weight under salt stress was proportional. In two varieties (OM90-9 and OM1630-50), however, the T/R ratio decreased significantly at a high salt level (16 dS m<sup>-1</sup>) as compared to the control, indicating that the reduction of shoot dry weight was severer than that of root dry weight. These findings are partly consistent with earlier reports, which showed that root growth was less affected than shoot growth under saline conditions<sup>12, 13, 17)</sup>.

The varieties exhibiting a high survival rate under salt stress also displayed a higher relative shoot growth than other varieties. However, the genotypic response to salt stress at the germination and seedling stages was not positively correlated. It was reported in the literature that the tolerance to salinity of rice plants varied with their developmental stages<sup>5, 10, 11, 16)</sup>. The salt-tolerant variety, Pokkali, showed a high germination rate

(85%) in salt solution, while Than Nong Do, also ranked as salt-tolerant, showed a low germination rate (25%) at the same salinity level. Some semi-dwarf varieties which showed high germination rates under salt stress were salt-susceptible at the seedling stage. This phenomenon can be attributed to differences in the plant response to salt stress at the developmental stages at which genes are operating after salt exposure<sup>8)</sup>. During the seedling stage, the salinity effect also varied with the age of the seedlings at the time of exposure to salt. The results of our studies showed that in both the tolerant and susceptible varieties, salinity effects were less severe on 15-day-old seedlings than on 6-day-old seedlings. Salt tolerance of the rice varieties increased with age, indicating that the seedling age was a critical factor in screening for salt tolerance, which is consistent with the report of Akbar and Senadhira (1988)<sup>4)</sup>.

Rice seedlings responded differently when they were exposed to different kinds of salts. Calcium chloride retarded the shoot growth more than other salts did, while Na<sub>2</sub>SO<sub>4</sub> was less detrimental. Tur *et al.*<sup>20)</sup> reported that rice plants were less tolerant to NaCl than to Na<sub>2</sub>SO<sub>4</sub>. In a study on wheat, CaCl<sub>2</sub> was also found to exert more adverse effects than NaCl<sup>1)</sup>. When ECs are adjusted to the same level, Cl<sup>-</sup> concentration in NaCl solution will be two-thirds of that in CaCl<sub>2</sub> solution, while Na<sup>+</sup> concentration in NaCl solution will be two-thirds of that in Na<sub>2</sub>SO<sub>4</sub> solution. Therefore, it is assumed that chloride ion is more toxic to rice seedlings than a equivalent sodium ion, because the growth was more significantly inhibited by calcium chloride than by sodium chloride. The response of the seedlings to different kinds of salts was similar in tall and semi-dwarf variety groups.

There was a difference in the degree of salt tolerance between tall and semi-dwarf variety groups. At high salt levels (above 12 dS m<sup>-1</sup>), no semi-dwarf varieties were tolerant to salt, except for OM997 (Fig. 1B). Plant vigor of a tall-type plant and its high survival rate under salt stress may be correlated. However, since the salt-tolerant

varieties often give a low yield, breeding efforts should aim at improving the salt tolerance in the semi-dwarf varieties with a high yield potential. There is no disadvantage *per se* in improved plant types, provided that the reduced stature can be compensated by selection for lower salt transport. In our studies, we identified a semi-dwarf variety (OM997) which was moderately salt-tolerant. This is a good example of breeding efforts in combining salt tolerance and high yield in a variety.

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## ベトナムの栽培イネ品種の耐塩性について

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### 摘 要

ベトナム由来の栽培イネ品種を用いて, 発芽期ならびに幼苗期における耐塩性を調べた結果, これらの二つの生育ステージにおける耐塩性の間に正の相関はみられなかった。15%の塩化ナトリウム溶液中での発芽率はMot Buiで最も低く(3%), OM1630-50で最も高かった(81%)。長稈品種と半わい性品種のそれぞれのグループの中でも, 耐塩性の強弱に大きな変異がみられた。長稈品種Thang Nong Doは, 耐塩性標準品種であるPokkali

と同程度の高い耐塩性を示した。半わい性品種OM997は, 中程度の耐塩性と高い収量性を合わせ持っていた。塩ストレスに対して, より若い苗ほどより高い感受性を示すことがわかった。また電気伝導度を同じにする種々の塩の影響を調べた結果, 塩化ナトリウムと比較して塩化カルシウムの害作用はより強く, 硫酸ナトリウムの害作用はより小さかった。

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