*Bot. Bull. Academia Sinica* 26: 97-104 (1985)

# *IN VITRO* IMPROVEMENT OF SALT TOLERANCE IN A RICE CULTIVAR[1,2]

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(Received, September 29, 1984; Accepted January 10, 1985)

**Abstract**

The research was aimed to maintain the genetic background of a cultivated rice Tainung 67 and to raise its level of salt tolerance. Seeds were treated with 0.025M ethyl methanesulfonate for four hours. Seed calli and regenerated plantlets were induced and differentiated on salt-stressed medium. The regenerated plants derived salt tolerant and non-tolerant progenies in seedling tests. A number of highly tolerant seedlings were rescued after two weeks of 1.0% salt screening and grown to maturity outdoor. The tolerant selections were subjected to continuous screenings in their subsequent generations.

**Key words:** Rice; salt tolerance.

## Introduction

Rice paddy fields along the coastal areas of Taiwan have been affected by high salinity because of the north air current with salt mist and under-ground water contaminated by sea water. To reduce the salt level in those fields is, at the present time, economically unfeasible. Rice cultivars resistant to salinity are therefore highly desirable. The incorporation of salt resistance could be encouraged by the practice of induced mutations. However, induced mutations at intact plant level require a large amount of space and resources, and often be subjected to growing seasons. Cellular selection of mutations has been advocated in recent years as an attractive alternative because of its time-space compressibility and selection specificity (Yoshida and Ogawa, 1983; Chaleff, 1983). In this study, we reported the responses of rice seedling progenies to induced salt stress after attempting *in vitro* selections in a donor cultivar.

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[1] Paper No. 288 of the Scientific Journal Series, Institute of Botany, Academia Sinica, Taipei, Taiwan, Republic of China.

[2] This work was financially supported by the National Science Council of the Republic of China.

## Material and Method

*Production of Putative NaCl-tolerant Plantlets*

*Oryza sativa* cv. Tainung 67, an improved *keng* (*japonica*) is highly popular among farmers throughout the island because of its high yield and wide adaptability and other desirable agronomic characters. Dehusked seeds of the cultivar were sterilized in 2.5% sodium hypochloride, soaked in water for 24 hours, and then mutagenized with 0.025M ethyl methanesulfonate (EMS) for 4 hours at 26±1°C. Mutagenized seeds were then subjected to another cycle of sterilization and rinsed in water before being placed on MS (Murashige and Skoog, 1962) medium, supplemented with 2 ppm each of 2,4-dichlorophenoxy-acetic acid (2,4-D) and kinetin for callus induction. Incubation conditions were in darkness and at 26±1°C. Two weeks later, induced calli were excised and allowed to proliferate on the original medium for further 10 days. They were then harvested, mechanically broken into crumbs and inoculated on a selection medium, which was identical to the medium for callus induction except that analytical grade of NaCl was incorporated at the level of 0.6, 1.0, 1.5 or 2.0%.

Callus crumbs that survived the selection process (about one month) and showed signs of good growth were isolated and transferred onto modified MS differentiation medium for regeneration into whole plants. The medium was supplemented with 0.2 ppm NAA and 2 ppm kinetin. NaCl at 0.6 or 1.0% was also incorporated in the regeneration medium. Cultures were incubated under a regime of 16 h light/8 h dark photoperiod at 26±1°C. When plantlets were about 15 cm tall, they were removed and hardened on nutrient solution before being transplanted in the field for obtaining selfed seeds.

*Screening of Progenies for Salt Tolerance*

For germination, growth or maintenance of progeny seedlings, a commercial plant food, Hyponex 7-16-19 (The Hyponex Co., Copley, Ohio, U.S.A.) was used at the level of 1 g/l. Different strengths of salinized nutrient solution were prepared by adding to the Hyponex nutrient solution with appropriate amount of unrefined crude sea salt. Thereafter, it would be referred to as salinized nutrient solution or simply salinized solution. The crude sea salt was obtained from Taiwan Salt Works, and contained at least 90% NaCl in addition to other components commonly present in sea water. Evaluation of salt tolerance was conducted in an unheated glasshouse in February-May 1983, with average temperature ranging 13 to 25°C during the period. Details of salinization treatment were as follows:

1. Test for possible maximum salt tolerance.

A total of 48 plant lines with good fertility were involved in the test. The parental plants of the progeny lines were obtained from seed calli preselected on

2% NaCl and regenerated on 1%. Two plump seeds from each lines were germinated directly in a test tube 1.2×10 cm containing 5 ml Hyponex nutrient solution. When seedlings were about 10 cm tall, they were treated with 2% salinized solution. Seedling height was recorded on the 8th day after salinization. The experiment was conducted in duplicates.

2. Response of seed germination and subsequent seedling growth to 3 salt doses.

A total of 2 seeds from each plant line was directly germinated and grown in a test tube containing 5 ml of 1.0, 1.5, or 2.0% salinized solution. The solution was renewed every two days and seedling height were studied on the 10th and the 14th day after salinization.

3. Salt treatment and seedlings.

A total of 20 seeds from each regenerated line was germinated in a transparent plastic vial 3×3.6 cm and grown until 5 cm tall. The seedlings were then subjected to 0.6% salinization for 10 days. Initial and subsequent seedling heights were then compared. Records of survivals were also taken; once on the 10th day and again on the 28th day after salinization. Survivals from the above salinization treatment were exposed for one further week to higher dose of 1% salt. Plants that survived the second imposition of stress were washed with water and allowed to recover for a few days before being transplanted in field.

4. Response of fully grown seedlings to 0.6% salinizatization.

Seedlings about 15 cm tall, 10 from each regenerated line, were subjected to 0.6% salt treatment for 18 days. The treatment was aimed to evaluate the effect of light salinization in a rather long period.

5. Response of seedling heights to 1% salinization.

Seedlings of 5, 10, 15, and 20 cm tall were treated with 1% salinized solution for 2 weeks. The treatment was aimed to determine the suitable seedling age for subjecting to salt stress.

## Results

### *Test for Possible Maximum Salt Tolerance*

Results of the test are given in Table 1. Since all the seedlings were derived from calli preselected and regenerated on salinized medium, it is reasonable to postulate that their progenies would also carry salt tolerance. However, the 2% salt treatment stunted all seedlings tested. Little growth was hardly found in 8 days, and all seedlings dried up in 14 days. This observation indicates that the concentration used was too severe for the testing purpose.

### *Response of Seed Germination and Seedling Growth to Three Salt Doses*

Results of the test are given in Table 2. A total of 49 plant lines was involved.

**Table 1.** *Seedling growth with 2% salt solution applied to approximately 10-cm tall seedlings*

| Range of growth (cm) | 0.0 0.5 | 0.6 1.0 | 1.1 1.5 | 1.6 2.0 | 2.1 2.5 | 2.6 3.0 | 3.1 3.5 | 3.6 4.0 | 4.1 4.5 | 4.6 5.0 | 5.1 7.5 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Regenerated line | 7 | 7 | | 16 | 1 | 5 | 4 | 3 | 1 | 2 | 2 |
| | 30 | | | | | 17 | | | | | |
| | control | | | | | | | | | | |

**Table 2.** *Segregation of seedling height (cm) for salt tolerance in the second generation of regenerated plants*

| Salt % | Days after germination | | | | | |
|---|---|---|---|---|---|---|
| | 10 | | | 24 | | |
| | control | | | control | | |
| 1.0 | 1.7-2.6<br>28*(57)** | 2.7-2.8<br>7(15) | 2.9-3.6<br>14(28) | 2.0-3.0<br>9(18) | 3.1-3.2<br>1(2) | 3.5-5.4<br>39(80) |
| 1.5 | 0.2-0.8<br>11(21) | 0.9-1.0<br>10(20) | 1.1-2.8<br>28(59) | 0.4-0.8<br>4(8) | 0.9-1.0<br>6(14) | 1.1-2.8<br>39(78) |
| 2.0 | 0.1-0.2 | 0.3-0.4<br>27(56) | 0.5-0.8<br>22(44) | No survival | | |

*, ** Number and per cent of regenerated lines.

For the 1% salt treatment, the seedlings of 14 plant lines (28%) grew faster than the control in the initial 10 days of the treatment. For the 1.5% salt, seedlings of 28 (59%) plant lines overgrew the controls. And, for the 2.0% salt, 22 (44%) plant lines performed well. On the 24th day after treatment, seedlings of 39 plant lines grew faster than those controls with 1.0 and 1.5% salts. This seems to indicate that the tolerance would be found only in a rather long period after being subjected to the salt treatment. No seedlings would survive in 2.0% salt for 24 days however.

*Young Seedlings Treated with Salt*

Seedlings of approximately 5 cm tall were subjected to 0.6% salt for 10 days. A total of 57 out of 89 plant lines overgrew the controls. However, most seedlings became stunted after a further 18 days on salinized solution and a portion of seedling leaves gradually dried up from their tips. Thus, the calculated growth became negative. The plotted graph of Fig. 1 revealed that the seedlings with rapid growth in the initial 10 days, usually grew rather slowly in the later 18 days. On the contrary, a number of plant lines showed a slow growth in the early 10 days; their growth picked up rapidly afterwards. A negative correlation,

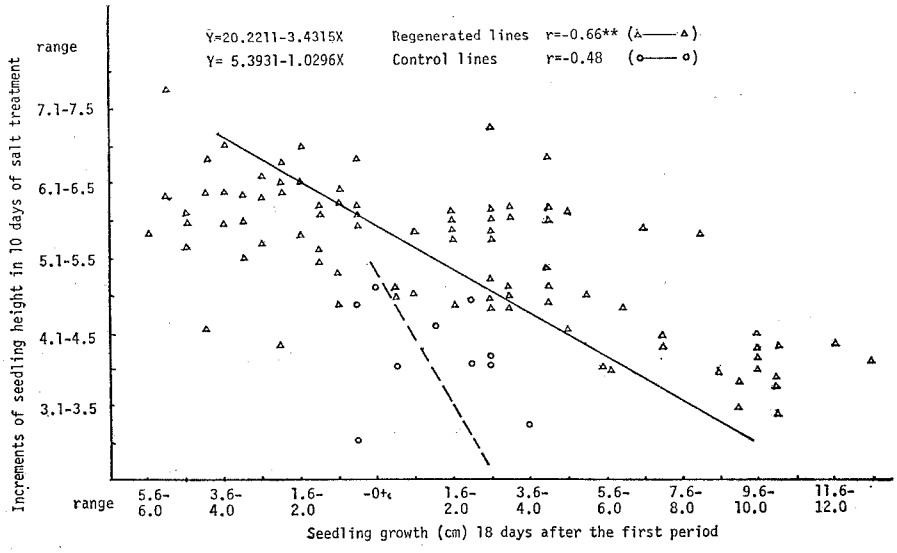


Fig. 1. Rice seedling growth with salt treatment of 0.6 percent.

$r=-0.66^{**}$ was found significant between the two arbitary periods. An insignificant correlation coefficient, $r=-0.44$ was found among the control lines between the two periods. The regenerated lines had viable progenies in salt tolerance compared with the controls, and 31 lines were identified as having improved tolerance. However, 32 plant lines had become more sensitive.

*Response of Fully Grown Seedlings to Salt*

Results of the test are given in Table 3. The seedlings of 15-cm tall were treated with 0.6% salt solution for 18 days. A total of 57 out of 95 plant lines showed better tolerance than the controls and 38 lines gave similar response of tolerance. The average heights of control seedlings were 13.2±0.7 cm and 22.3±1.4 cm before and after the salt treatment.

**Table 3.** *Seedling growth with 0.6% salt for 18 days*

| Range of growth (cm) | 3.1* 3.5 | 3.6 4.0 | 4.1 4.5 | 4.6 5.0 | 5.1 5.5 | 5.6 6.0 | 6.1 6.5 | 6.6 7.0 | 7.1 7.5 |
|---|---|---|---|---|---|---|---|---|---|
| Regenerated line | 1 | 10 | 14 | 13 | 15 | 18 | 18 | 5 | 1 |
| | | | 38 | | | | 57 | | |
| control | | | | | | | | | |

* Increments in cm after salt treatment.

*Response of Different Seedling Height to 1% Salt*

Results of the test are given in Table 4. Seedlings averaged approximately 5 cm survived by 20% after being subjected to salt for two weeks. The number of survivals per plant line varied from 1 to 10 seedlings. The average growth was estimated at 6.8 cm. The rather old seedlings of 11.6±1.3 cm tall survived by 25.5% after salt stress. The number of viable seedlings per plant line was only 1.8±0.8 while the rest of seedlings dried up and died in two weeks. Seedlings averaged of 14.5-cm tall did not survive well, only 5.5% plant lines having 1 to 2 viable green seedlings. However, the fully grown seedlings approximately 20-cm tall survived well under salt stress for two weeks. Approximately 62.5% plant lines survived by 1 to 10 seedlings, though the growth of the seedlings stunted and necrosis appeared on leaf tips. Therefore, a negative growth of −0.7±2.2 cm was found. Since the growth variation was highly apparent, the standard deviation was higher than the mean however. This result also elucidates that the variation of tolerance among plants was considerably large. The survivals would carry certain level of tolerance.

**Table 4.** *Response of plants to 1% salt at four seedling heights*

| Line | Height (cm) | Number of survival | | | | Growth (cm) |
|---|---|---|---|---|---|---|
| | | Lines | % | Seedling | Average | |
| 145 | 4.8±0.8* | 29 | 20.0 | 1-10 | 2.8±2.3 | 6.8±1.6 |
| 〃 | 11.6±1.3 | 37 | 25.5 | 1- 4 | 1.8±0.8 | 0.1±4.2 |
| 144 | 14.9±1.8 | 8 | 5.5 | 1- 2 | 1.3±0.4 | 2.8±1.2 |
| 〃 | 20.6±2.6 | 90 | 62.5 | 1-10 | 3.6±2.8 | −0.7±2.2 |

* Values are quoted as the mean ±S. E.

## Discussion

Cultivars with salt tolerance may widen their adaptability to coastal area having saline soil. This trait of economic potential highly interests researchers of diverse disciplines. Several mechanisms of salt tolerance were early reviewed by Rains (1972). Later, Dix and Street (1975) obtained tobacco cell cell line that would survive on 1.0-2.0% NaCl salinized medium. Nabors *et al.* (1975, 1978, and 1980) made a series of studies on tobacco. They reported that the tolerant cells could survive well on 0.88% salt compared with those of control of 0.16%. Further, Croughan *et al.* (1978) and Croughan (1981) selected an alfafa cell line tolerant to 1.0% NaCl. The cell line could even grow better on NaCl-stressed medium than on those without added NaCl. These works were somewhat prior to our attempt of raising the level of salt tolerance without interrupting the major genetic structure

of rice stock. Thus, the plant/cell lines developed from the research might have commercial potentials besides being used for basic analysis of salt tolerance.

Stone *et al.* (1979) reported that the salt tolerance was associated with temperature. Tolerance may be reduced or broken down with the rising of temperature. Our seedling screening for salt tolerance was conducted in an unheated glasshouse starting from early spring 1983. The first batch of seedlings was grown in early spring under rather low temperature. The slow growing seedlings reacted slowly to the induced salt stress. The effect of salt toxicity did not become apparent until two weeks after treatment. However, symptoms could be readily observed in one week under high temperature of over 30°C. The differential response may have been caused by the presence of roots and the less need for transpiration under low temperature. Thus less amount of water absorption from stressed solution will be needed to fit the need of plant transpriation. On the contrary, seedlings raised under high temperature of over 30°C; plant transpiration is intensive. Therefore, the interruption of water absorption to be less functioning by salt stress would make the plants becoming sensitive to transpiration.

Unrefined crude sea salt instead of pure NaCl was used in screening for salt tolerance because of it more resembles the conditions to which plants are exposed in nature. The crude salt is known to contain a number of minor amount of salts other than NaCl. Similar study was attempted by Chen *et al.* (1980). Their tobacco calli were found to be better grown on crude salted medium than on those of NaCl stressed. They concluded that the absorption of $Mg^{2+}$, $K^{+}$ and $Ca^{2+}$ ions would reduce the toxicity of $Na^{+}$ ions. Tal *et al.* (1978) reported that wild tomatoes were highly sensitive to NaCl, mannitol, proline and its analog 3,4-dehydroproline or combination of these substances compared with the cultivated ones. The wild species have well been adapted to the natural habitat; probably a great number of salts existed in small amount. On the other hand, the cultivated crop have been usually subjected to heavy chemical fertilization. Maybe, the fertilization would result in cultivated tomato becoming insensitive to chemical salts. Their findings seem likely to interpretate that most plants, in general, may become tolerant to mixture of salts rather than a single one, especially the NaCl. It also elucidates that the crude sea salt could well suit the screening practice for salt tolerance. Once tolerant plants are identified, and if their original genetic background are preserved, they could be gradually accepted for commercial production.

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# 水稻耐鹽性之改進與培養

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本研究之目的，在應用組織培養方法，提高水稻之耐鹽力，又不影響品種之遺傳結構。粳稻品種臺農67號之種子先用 EMS, 0.025M 浸種處理4小時，種子之癒合組織及再生小株均得自含鹽之培養基。再生植株之後代，部份系統具有不同層次之耐鹽力，亦有不具耐力。秧苗均用1%鹽之培養劑培養兩星期，仍能生存之秧苗，經洗鹽後移植田間，後代種子作進一步之研究。