Screening rice for salinity tolerance

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Breeding for salinity tolerance in rice requires reliable screening techniques. These techniques must be rapid to keep pace with the large amount of breeding materials generated. Screening under field conditions is difficult due to stress heterogeneity, presence of other soil-related stresses, and the significant influence of environmental factors such as temperature, relative humidity, and solar radiation. These complexities, together with the degree of control of salinity and reproducibility, cause difficulties in developing and using reliable methods of screening voluminous materials.

Rice is one of the most suitable crops for saline soils although it is usually considered moderately sensitive to salinity (Akbar et al 1972, Korkor and Abdel-Aal 1974, Maas and Hoffman 1977, Mori et al 1987). Saline soils are usually under waterlogged condition; other crops could not grow in these areas except rice. Salt tolerance is generally a sustained growth of the plant in the soil environment impregnated with NaCl or other salt combinations.

Earlier studies conducted under controlled conditions reported that salt injury in rice plant is caused by both osmotic imbalance and accumulation of chloride (CI) ions (Iwaki et al 1953, Shimose 1963). Recent studies, however, indicated that injury is due to the excessive sodium (Na) uptake, and chloride, being essentially a neutral anion, is tolerated over a wide range of concentrations (Clarkson and Hanson 1980). The

disruptive effect of Na and its interference with the role of cytoplasmic K preempted CI toxicity. Moreover, Na-K imbalance adversely affects grain yield (Devitt et al 1981). The typical mechanism of salinity tolerance in rice is the exclusion or reduction of Na uptake and increased absorption of K to maintain a good Na-K balance in the shoot.

The detection of salinity induced injuries, however, are very complex even under controlled conditions. Moreover, it requires expensive and time-consuming tissue analysis. The visual symptoms of salt stress may still be the most appropriate for mass screening. Salt injury starts with reduction in effective leaf area. The oldest leaves start to roll then die, followed by the next older, and so on. Finally, the survivors have the old leaves losing vitality with the youngest remaining green.

Several studies indicated that rice is tolerant during germination, becomes very sensitive during early seedling stage (2-3 leaf stage), gains tolerance during vegetative growth stage, becomes sensitive during pollination and fertilization, and then becomes increasingly more tolerant at maturity (Pearson et al 1966, IRRI 1967). However, some studies reported that at flowering, rice is not sensitive to salinity (Kaddah et al 1975). Hence, to know the response of the rice plant to salinity as a whole, it is imperative that the effects be observed in all the various stages of its development, that is at early seedling, vegetative and reproductive stages.

A screening method for salinity tolerance could be readily acceptable when based on a simple criterion for selection, it provides rapid screening of large number of materials and reproducible results. In Plant Breeding, Genetics and Biochemistry

Division (PBGB), IRRI, two screening techniques were developed for use at seedling stage, and vegetative and reproductive stages. Screening is done under controlled condition to minimize environmental effects.

SCREENING AT SEEDLING STAGE

Rice is very sensitive to salinity at seedling stage. Its height, root length, emergence of new roots, and dry matter decrease significantly at EC (electrical conductivity) 5-6 dS m⁻¹ (Pearson et al 1966, Akbar and Yabuno 1974). Salinity stress at early seedling stage manifest on the first leaf, followed by the second, and finally on the growing leaf. Salinity suppresses leaf elongation and formation of new leaves. Photosynthetic function and chlorophyll content were inversely proportional to salinity level (Ota and Yasue 1962). The screening technique developed is based on the ability of seedlings to grow in salinized nutrient solution.

Materials and instruments needed

To conduct the screening technique at seedling stage, one will need some basic items of equipment and materials. Given below is the list of items that are basic minimum for setting up the screening technique. Simple laboratories usually have most of the equipment mentioned (e.g. pH meter, balances, convection oven, magnetic stirrers, etc.). Only very few items may be needed to complete the setup.

- pH meter
- EC meter

- Balance (1000-g capacity and 0.0001-g readability)
- Magnetic stirrer
- NaOH and HCI
- NaCl (analytical grade)
- Reagents (analytical grade) for nutrient solution (see Table 1)
- Glass bottles
 - 6 pieces, 1-L capacity
 - 12 pieces, 2.5-L capacity (dark glass)
- Convection oven
- Germinator oven
- Volumetric flasks: 100- and 200-mL capacity
- Graduated cylinders: 25, 50, and 100 mL

Plastic trays: 12-L capacity rectangular trays of size 14 x 30 x 35 cm

Dark color trays are preferred.

- Beaker: 1000 mL
- Styrofoam sheets (1.25 and 2.5 cm thick for making seedling floats)
- Nylon net (insect proofing type)
- Mixing containers: Cylindrical plastic containers, 50-L and 100-L capacity

Making of seedling floats

The setup requires styrofoam seedling floats. A float is a fabrication of a rectangular styrofoam of size $28 \times 32 \times 1.25$ cm having 100 holes (10 x 10) with nylon net bottom and 2.5 cm thick frame pasted on top. The frame helps fit the float to a rectangular plastic tray with 12-L capacity and 4-x-30-x-35-cm size. For detailed fabrication, cut a rectangular piece of 1.25 cm thick styrofoam size 28×32 cm. Drill holes with about 1 cm diameter in rows of 10 (see Fig. 1). Attach a piece of nylon net to one side and stitch with nylon thread. Cut a 2-cm thick styrofoam frame (31 x 36 cm) as shown in Figure 1. The inside of the frame is about 1 cm smaller than the rectangular piece on all sides. This area is used to paste the rectangular piece to the frame. Once pasted, it should fit well to the top of the plastic tray.

Preparation of stock solutions

Proper preparation of stock solutions is essential to avoid nutrient deficiencies and mineral toxicities not attributed to salinity stress. It is advisable to prepare fresh stock solutions every two months. Therefore, the amounts prepared should depend on the number of test entries screened during a two-month period. If 30 trays are used continuously the amount of stock solution needed for each element and for a two-month period is approximately 4 L. Table 1 gives details for preparing 4-L stock solutions. For the macronutrient stock solutions, weigh the required amount of reagent (see Table 1) and transfer to a 1000-mL beaker and do initial mixing with about 750 mL distilled



Fig. 1. Seedling float for salinity screening at the seedling stage.

water. Pour the mixture to the 2-L volumetric flask, then add distilled water and make up volume to 2 L. Stir the mixture for 15 min using a magnetic stirrer then transfer to stock solution bottle.

Preparation of micronutrient stock solution is critical because most nutrient deficiencies and other toxicities could be traced to improper preparation. Thus micronutrient preparation must be given considerable attention. Each reagent of the micronutrient solution listed in Table 1 should be dissolved separately. Use 50 mL distilled water to dissolve each reagent except for ferric chloride, which must be dissolved in 100 mL distilled water. Mix all solutions together in 1 L distilled water using 2.0-L capacity volumetric flask. Add the ferric chloride solution to the mixture just before citric acid and stir the mixture for 15 min using a magnetic stirrer. Finally add 100 mL sulfuric acid to the mixture and make up volume to 2.0 L. Stir for another 10 min and store in a dark glass bottle. The final color of this solution must be yellowish brown. All stock solutions must be properly labeled and kept in separate bottles.

Salinization of nutrient solution

Salinize the nutrient solution by adding NaCl while stirring up to the desired EC (3 and 6 g NaCl L⁻¹ nutrient solution gives an EC of 6 and 12 dS m⁻¹ respectively). Fill up the trays with this solution high enough to touch the nylon net bottom of the styrofoam. The effective culture solution needed per tray is about 11 L. However, it is practical to prepare more than the exact amount in case of spillage.

Element	Reagent (AR grade)	Preparation (g / 4 L solution)
Macronutrient		
Ν	Ammonium nitrate (NH ₄ NO ₃)	365.6
Ρ	Sodium phosphate, monobasic monohydrate (NaH ₂ PO ₄ .H ₂ 0)	142.4
К	Potassium sulfate (K ₂ S0 ₄)	285.6
Са	Calcium chloride, dihydrate (CaCl ₂ .2H ₂ 0)	469.4
Mg	Magnesium sulfate, 7-hydrate (MgSO ₄ .7H ₂ O)	1296.0
<u>Micronutrient</u>	Dissolve each reagent separately and n then add 200 mL H ₂ SO ₄ and make up	nix in 2 L distilled water volume to 4 L
Mn	Manganous chloride, 4-hydrate (MnCl ₃ .4H ₂ O)	6.000
Мо	Ammonium molybdate, 4-hydrate [(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O]	0.296
Zn	Zinc sulfate, 7-hydrate (ZnSO ₄ .7H ₂ O)	0.140
В	Boric acid (H ₃ BO ₃)	3.736
Cu	Cupric sulfate, 5-hydrate (CuSO ₄ .5H ₂ O)	0.124
Fe	Ferric chloride, 6-hydrate (FeCl ₃ .6H ₂ O)	30.800
	Citric acid, monohydrate (C ₆ H ₈ O ₇ .H ₂ O)	47.600

Table 1. Preparation of stock solution.

Source: Adapted from Yoshida et al (1976) Note: For easy handling and storage, hydrated reagents are preferred.

Element	Reagent	mL of stock solution / 360 L nutrient solution	Concentration of element (ppm)
Macronutrient			
N	NH ₄ NO ₃	450	40
Ρ	NaH ₂ PO ₄ .2H ₂ O	450	10
К	K ₂ SO ₄	450	40
Са	CaCl ₂ .2H ₂ O	450	40
Mg	MgSO ₄ .7H ₂ O	450	40
Micronutrient			
Mn	MnCl ₂ .4H ₂ O	\mathbf{i}	0.50
Мо	(NH ₄) ₆ .Mo ₇ O ₂₄ .4H ₂ O		0.05
Zn	ZnSO ₄ .7H ₂ O	450	0.01
В	H ₃ BO ₃	450	0.20
Cu	CuSO ₄ .5H ₂ O		0.01
Fe	FeCl ₃ .6H ₂ O)	2.00

Table 2. Element composition of nutrient solution.

Source: Adapted from Yoshida et al (1976)

Management of nutrient solution

The maintenance of the nutrient solution is very important. Considerable attention has to be given in adjusting the pH. Monitoring and maintaining the pH of the culture solution is very critical because this checks the balance of the available nutrients. Significant deviation (\pm 1.0) of culture solution pH from 5.0 will make some nutrients toxic and others deficient, thus a reliable pH meter and its regular calibration are essential.

Since ammonium and nitrate are the sources of nitrogen, the pH will decrease during the first few days because ammonium ion is favorably absorbed by the plant than nitrate ion. The pH will then increase when ammonium ion is depleted and more nitrate ion is taken up by the plants. The increase of the pH in the nutrient solution could be used as an indicator that nitrogen source starts to be deficient. Change the nutrient solution every 8 d.

It was mentioned earlier that dark colored trays are preferred. This is because any light penetration into the culture solution promotes algal growth. Algae are not harmful but they tend to increase pH particularly during mid-day and early afternoon. If substantial algal growth is observed, it is advisable to adjust the pH twice a day, for example at 0900 and 1500 h. Due to evaporation and transpiration there will be loss of solution volume in the trays. Make up the volume with distilled water every 2 d.

Calibration of pH and EC meters

Maintaining the pH of the culture solution is very critical. Any error in the pH may create unnecessary stress to the plants due to toxicity and/or deficiency of nutrients and not because of salinity. Calibration of pH meter must therefore be regular. Use two buffers in calibrating at pH 4 and 7. These must be done at least once a week. Spare pH electrodes are essential. The EC meter measures the amount of electrolytes present in the solution. The amount of electrolytes is associated with the degree of salinity. The EC of the solution will not change significantly from day 1 to about day 8. Errors in EC reading from the meter could be easily detected because pre-measured amount of NaCI should register the expected EC level (6 g NaCI L⁻¹ gives an EC of 12 dS m⁻¹).

However, calibration of the EC meter should be done at least once a month using the 7.01 dS m⁻¹ buffer.

Handling of seedlings and salinization

Test seeds have to be heat-treated for 5 d in a convection oven set at 50 °C to break seed dormancy. Proper breaking of the seed dormancy is very essential in this screening technique. Delay in germination of some entries will likely make these entries more sensitive to salt. Seedling vigor has great advantage at this point since salinization occur at very early seedling stage. After breaking the dormancy, surfacesterilize seeds with fungicide and rinse well with distilled water. Place sterilized seeds in petri dishes with moistened filter papers and incubate at 30 °C for 48 h to germinate. Sow two pregerminated seeds per hole on the styrofoam seedling float (Fig. 1). The radicle should be inserted through the nylon mesh. During this process, the radicle may be damaged and this damage may not be visible. Any damage to the radicle will destroy the main salt tolerance mechanism of rice. Therefore, sufficient time should be allowed for the seedlings to repair any root damage. For this reason, pregerminated seeds should not be seeded in salinized nutrient solution. Suspend the styrofoam seedling float on the tray filled with distilled water. There are adequate nutrients in the endosperm for the seedlings to grow normally for 3-4 d. After 3 d, when seedlings are well established, replace the distilled water with salinized nutrient solution. Initial salinity is at EC = 6 dS m⁻¹. Three days later, increase salinity to EC=12 dS m⁻¹ by adding NaCI to the nutrient solution. Renew the solution every 8 d and maintain the pH at 5.0 daily. Test entries can be rated at 10 and 16 d after initial salinization.

At IRRI, this test is conducted in phytotron glasshouse maintained at 29 °/21 °C day/night temperature and minimum relative humidity of 70% during the day. These controlled conditions minimize environmental interactions. However, ordinary glasshouses or greenhouses could also be used. Please see section on possible modifications.

Test entries and checks

The seedling float has 10 rows with 10 holes each. One row could be used for one test entry. It is essential to have check varieties in every seedling float to guide in rating the visual symptoms of salinity stress. At IRRI, we use three check varieties--IR29, sensitive; IR74, moderately tolerant (both improved varieties from IRRI); and Pokkali, tolerant (traditional variety from India). With these checks, 7 test entries can be screened in one seedling float on a tray.

Evaluation of salt stress symptoms

Use the modified standard evaluating score (see Table 3) in rating the visual symptoms of salt toxicity. This scoring discriminates the susceptible from the tolerant and the moderately tolerant genotypes. Scoring may start at 10 d after salinization and final scoring at 16 d after salinization. At 10 d after salinization, Pokkali scores: 1; IR74: 5; and IR29: 7. During this time, susceptible genotypes could be distinguished from the test entries. However, tolerant types could not be readily identified from the moderate ones. At 16 d after salinization Pokkali scores: 3; IR74: 7; and IR29; 9. By this time,

there will be a clear distinction among the test entries: the tolerants, moderates, and the susceptibles.

ScoreObservationTolerance1Normal growth, no leaf symptomsHighly toleran3Nearly normal growth, but leaf tips or few leaves whitish and rolledTolerant	
1Normal growth, no leaf symptomsHighly toleran3Nearly normal growth, but leaf tips or few leaves whitish and rolledTolerant	
3 Nearly normal growth, but leaf tips Tolerant or few leaves whitish and rolled	t
5 Growth severely retarded; most leaves Moderately to rolled; only a few are elongating	lerant
7 Complete cessation of growth; most Susceptible leaves dry; some plants dying	
9 Almost all plants dead or dying. Highly suscep	tible

Table 3.	Modified standard evaluation score (SES) of visual salt injury at seedling
	stage.

Possible modifications

Plant analysis. This screening technique could be used to determine Na and K concentration in shoot and then derive the Na-K ratio as an indicator for tolerance. This could be done with some modifications in the timing of salinization. If salinization begins 3 d after seeding, the susceptible check will be dead in about two weeks and the amount of root and shoot will not be sufficient for tissue analysis. It is advised that plants are grown up to 14 d in normal nutrient solution to allow sufficient growth before salinization. In this case, seeding could be done in nutrient solution instead of distilled water. Salinization can start 14 d after sowing with EC of 12 dS m⁻¹. In this modification, addition of silicon in the form of sodium meta-silicate 9-hydrate (4.5 mg L⁻¹)

of culture solution) is essential to avoid lodging of plants. Silicon strengthens the shoots of the test entries enabling the plants to withstand the disturbance due to the daily pH monitoring. Sampling is done when the susceptible check scores 7, that is about 25-30 d of salinization. Some of the dead leaves may drop off during pH adjustment and renewal of culture solution. They should be collected and included in the final sample. There should be some greenness (youngest leaf or the stem) in the sensitive check during sampling. If all leaves are dried up, there is no Na entry to the plant but it occurs in other entries with green leaves and thus leads to wrong comparisons. For tissue analysis, collect the shoot samples per entry and wash carefully with distilled water especially the lower part where it may contact with the salinized culture solution. Oven-dry the shoot samples for 3 d at 70 °C. Grind dried samples to obtain about 1 g (40 mesh) powdered form from each sample and analyze Na and K concentrations using atomic absorption spectrophotometry.

Use of greenhouse. Another possible modification is the use of greenhouse instead of Phytotron. This would make the screening method easily adaptable to many situations. In the greenhouse, temperature and relative humidity will vary. Adverse effects of salinity decrease with low temperature and high humidity. Solar radiation is another factor to consider. Increase in solar radiation increases the stress. Taking these into consideration, the EC level or the duration of stress should be adjusted. The best indicator for any adjustment is the response of check varieties. If the stress is low due to high humidity, or low temperature and solar radiation, it is better to extend the stress period than increasing the EC level.

Distilled water. If distilled water is not available in large quantities, demineralized water could be used to prepare the nutrient solution.

Salinization. Some researchers may like to include other salts such as calcium chloride for salinization. This is acceptable. We have used different ratios of NaCI : CaCl₂ in some of our tests. However, as you increase the ratio, some symptoms could appear that may not be due to NaCI toxicity but to calcium toxicity. Such symptoms were observed at 8:1 NaCI-CaCl₂ ratio.

Seeding. If the test is conducted in a greenhouse where air temperature is usually high (specially during dry season), the seeds, after sterilization could be soaked for 24 h and sown for germination. Distilled water or nutrient solution could be used as the initial growing medium.

Checks. It is possible to use only two checks: sensitive and tolerant, to increase the number of test entries. However, we advise the use of all three checks -- tolerant, moderately tolerant, and susceptible -- at the beginning or until the researcher gains competence in rating test entries.

Test entries. We use 20 seedlings (10 holes) for screening early generation pedigree lines. For very advanced pedigree lines and varieties, we use only 10 seedlings (5 holes). This enables us to test 17 entries (excluding checks) in one tray. The checks could also be reduced to 10 seedlings. Segregating populations such as F_2 and F_3 could also be screened using two seeds per hole. At IRRI, we do not screen such populations since our genetic studies (Gregorio and Senadhira 1993) have shown that it is not effective. We advance populations under normal or saline soil conditions (in the field) with minimal selection or by single seed descent (SSD) and advanced

bulks (F_5 or above) are tested by this method to isolate tolerant genotypes. After screening, the tolerant genotypes are carefully removed from the nylon mesh and are transferred to pots filled with normal soil where they mature. Testing 300 lines per month is possible and with only 2 working hours a day on the average.

Some results

Since 1990, we have successfully used this method for screening germplasm and breeding populations. Whenever Phytotron space is not available, the greenhouse was used. Because of high day temperature, stress level increased when conducted in the greenhouse but adjusting salinity level to EC 10 dS m⁻¹ minimized the problem associated with high temperature. However, we found that it is easier to discriminate susceptible from highly tolerant types in the greenhouse test. Moderately tolerant types may show susceptibility in greenhouse test.

To confirm the reliability of this screening technique, the visual salt-injury symptoms were compared with the actual grain yield of some test entries obtained in the field under saline and normal soil conditions. Table 4 shows these comparisons. The score based on visual symptoms relates well to grain yield under saline field condition and yield reduction due to salt stress. This shows the reliability of visual symptoms of salt stress after 16 d of salinization as the selection criterion for rapid screening of voluminous breeding materials.

 Entry	Salinity Score	<u>Yield (t ha⁻¹)</u> Saline Normal		Percent yield reduction
 IR29	9.0	1.74	6.29	72.34
IR9884-54-3-1E-P1	3.0	3.03	7.37	58.89
IR51500-AC11-1	3.0	3.18	7.58	58.05
IR45427-2B-21-2B-1-1	3.0	2.46	7.81	68.50
IR52717-2B-9-2B-1-2	3.5	2.21	5.14	57.00
IR52713-2B-9-2B-1-2	5.0	2.29	6.98	67.19
IR52709-2B-5-2B-1-1	7.0	1.94	6.96	72.13

 Table 4.
 Salinity rating under salinized culture solution and grain yields under saline and normal soil farmer field conditions.

The typical mechanism of salinity tolerance in rice is the Na exclusion or uptake reduction, and increased absorption of K to maintain a good Na-K balance in the shoot. The mean performance for salinity rating, Na⁺, K⁺ and Na-K absorption ratio in the shoots of indica rice are presented in Table 5. These data demonstrate the high correlation (r = 0.93) between Na-K ratio and salinity tolerance score. The established classification of susceptible, moderate, and tolerant based on field, laboratory, and greenhouse tests was clearly related to visual injury symptom rating as well as Na-K ratio. On the basis of absorbed Na⁺, susceptible line M1-48 could be classified as being moderately tolerant. With respect to K⁺ absorption, tolerant entry Pokkali demonstrated moderately tolerant behavior while the moderately tolerant line IR9884-54-3-1E-P1 exhibited tolerant behavior. Thus ranking according to Na⁺ or k⁺ absorption

alone is not a reliable parameter for salinity tolerance reactions. However, the classificaiton of susceptible, moderately tolerant, and tolerant based on field, laboratory, and greenhouse tests is clearly related to the Na-K ratio. This Na-K ratio, which is the balance between Na⁺ and k⁺ in the shoot, could then be a valid criterion in measuring salinity tolerance in rice. The same trend was observed in japonica rices (Lee 1995). Na-K ratio could then be a good parameter in quantifying the degree of salinity tolerance for genetic and molecular studies that are now pursued at IRRI using this screening technique. Determination of Na-K ratio is tedious and expensive. Visual symptom rating is adequate to determine the level of tolerance for breeding purposes. Using this technique, the degree of tolerance of large number of genotypes is determined in 3 wk. Visual symptom rating correlated well with yield performance in saline rice fields. This technique could accelerate rice breeding programs for salt tolerance.

Entries	Salinity score	Na	К	Na-K ratio	Reaction to salinity
IR28	7.0	0.652	1.935	0.359	Susceptible
IR29	7.0	0.835	2.410	0.350	Susceptible
M1-48	6.0	0.582	2.080	0.284	Susceptible
IR4595-4-1-13	4.5	0.510	2.435	0.209	Moderate
IR9884-54-3-1E-P1	4.5	0.526	2.630	0.200	Moderate
IR10206-29-2-1	4.8	0.599	2.295	0.261	Moderate
Nona Bokra	3.0	0.456	2.540	0.180	Tolerant
Pokkali	3.0	0.397	2.480	0.159	Tolerant
SR26B	3.5	0.452	2.850	0.159	Tolerant

Table 5.Salinity score and mean of Na, K and Na-K ratio in shoot of some indicarice grown under salinized culture solution.

SCREENING AT VEGETATIVE AND REPRODUCTIVE STAGES

Determining the level of salinity tolerance during vegetative and reproductive stages is difficult. Rice plant gains tolerance to salinity at reproductive stage. Plant height, root length, tillering ability, and biomass decrease when affected by salinity. At reproductive stage, salinity causes an increase in sterile florets by affecting panicle initiation, spikelet formation, fertilization, and germination of pollen. Salinity also reduces panicle length, number of primary branches and spikelets per panicle, fertility and panicle weight, thus reducing grain yield (Pearson 1961, Akbar et al 1972).

Field tests are less reliable due to stress heterogeneity and other environmental stesses. Available laboratory and greenhouse tests are very expensive and time consuming. The salinity screening technique at vegetative and reproductive stages we developed is simple and reliable. The technique requires a well ventilated glasshouse. Ventilation is essential to reduce high afternoon temperatures usually experienced in glasshouses in the tropics. If glasshouse is not available, similar facility with natural day light and protection from rain could be used.

Materials and instruments needed

- Plastic pots (height = 15.0 cm, diameter = 11.0 cm)
- Plastic trays (any size with at least 22.0 cm height)
- Cloth bags (100% cotton)
- Table salt (technical grade)
- Electrical Conductivity meter
- Well ground soil, preferably from paddy field
- Fertilizer (N, P, and K)

Preparation of pots and cloth bags: The wall of plastic pot should be drilled with 3-4 mm diameter holes 2 cm apart (Fig. 2). An electric bouth could be used to drill holes. The top most circle of holes should be at least 3 cm below the rim of plastic pot. Using 100% cotton cloth, stitch bags that fit well inside the plastic pot. These cloth bags can be used only once.



Fig. 2. Setup for salinity screening at vegetative and reproductive stages.

Seeding

Place a cloth bag inside the pot and fill with fertilized soii. At IRRI, rice field soil fertilized with 50N, 25P, and 25K mg kg⁻¹ of soil is used. Initially, the soil level should be about 1 cm above the topmost circle of holes. With leveled soil place pots in the plastic tray filled with ordinary tap water. This serves as water bath. The water level should be same as the soil level. The soil will then begin to settle as it absorbs water. While maintaining the water level, extra soil can be added after a day or two if necessary, to obtain the correct soil level. During this soil settling process, pregermination of seeds could start.

Four to five pregerminated seeds of test entries are placed on soil surface of each pot. Two weeks after seeding, seedlings should be thinned to three per pot and the water level be raised to about 1 cm above soil. Maintain water level daily and protect the plants from any pests and diseases.

Salinization

When the seedlings are 21 d old, siphon out all water in the water bath. Allow a 12-h break before siphoning out water that drains out from pots. Prepare salinized water solution up to the desired EC level by dissolving table salt (NaCl) in water while stirring. With the table salt used at IRRI, 4.5 g in 1 L give an EC of 8 dS m⁻¹. Fill up the plastic tray with salinized water solution. The water level must be maintained daily (1 cm above soil surface) by adding ordinary water and **not salinized water**.

Salinity level used at IRRI is 10.0 dS m⁻¹ in wet season and 8.0 dS m⁻¹ in dry season. Salinity of the water bath will gradually decrease and stabilize at around 2-3 EC units less than the original (Figs. 3, 4). The soil salinity level is more than the water bath and climatic conditions seem to influence the degree of increase. In wet season, the EC stabilizes at around 9 dS m⁻¹ (Fig. 3) while it is about 11 dS m⁻¹ (Fig. 4) in dry season. If the salinity level needs to be increased or decreased during the experiment, it can be done by draining all the water and replacing with water salinized to the desired EC level.

Fertilizer topdressing could be done if necessary. Apply fertilizers to soil and not to water bath. The plants should be maintained free of any pests and diseases. Usually, there are less pests and diseases in glasshouses but it is advisable to adopt prophylactic measures to ensure that salinity will be the only stress to the plant.

About 5-6 wk after seeding, roots growing into the water bath through the holes in plastic pot may be observed. Therefore the pots should not be disturbed because any damage to roots will interfere with the plant's mechanism of salt tolerance.

Test entries

The test entries must be grouped according to plant height and duration to have uniform conditions from flowering to ripening and to minimize shading effects. At IRRI, test entries are grouped into tall and semidwarf for height and, early (100 \pm 10d), medium (125 \pm 10d), and late (150 \pm 10d) for duration, resulting in 6 groups -- early-tall, early-semidwarf, medium-tall, medium-semidwarf, late-tall, and late-semidwarf.

Electrical conductivity (dS m⁻¹)



Fig. 3. Electrical conductivity of water bath and soil in wet season.



Fig. 4. Electrical conductivity of water bath and soil in dry season.

Checks

Tolerant and sensitive checks must be used for comparison with the test entries. It is advisable to include both checks in every tray. Suggested check varieties for every entry group are shown in Table 6.

Variety group		Tolerant check	Sensitive check	
Early				
	tall	TCCP266-1-3B-10-3-1	PP2462-11	
	semidwarf	IR45427-2B-2-2B-1-1	IR58	
<u>Mediu</u>	<u>um</u>			
	tall	Pokkali	FR13A	
	semidwarf	IR63731-1-1-4-3-2	IR29	
Late/	photosensitive			
	tall	SR26B	Wagwag	
	semidwarf	IR52724-2B-6-2B-1-1	Bg 407	

Table 6.Check varieties for salinity tolerance screening at vegetative and
reproductive stages.

Replication and randomization

Since only one test entry is planted in a pot, each pot could be considered as one experimental unit. The three seedlings are sub-samples. Either completely

randomized (CRD) or randomized complete block (RCB) designs could be used. For RCB, one tray or a set of trays can be taken as a block. We find that if grouping is done as described earlier, CRD is adequate to get good information.

Scoring for tolerance

The susceptible check will express stress symptoms (leaf rolling and whitening) in about 3 wk after salinization. Test entries can be scored from then onwards based on visual symptoms. Percent green leaf area is convenient and reliable to use as a measure of tolerance. It is suggested that entries are scored every 2 wk.

Grain yield per plant or pot is the best score for tolerance. Data based on the number of spikelets, filled spikelets, and unfilled spikelets could be collected but they may produce misleading results. It is impossible to get a good estimate of the total number of spikelets. Even in some tolerant varieties, some spikelets are malformed or aborted and sometimes they drop off before good spikelets mature.

The technique is used at IRRI for selection of advanced lines. Results obtained from one screening set are summarized in Table 7. This sample screening result was an evaluation of medium-semidwarf variety group. Based on grain yield per pot, salt tolerant lines could be detected. Most of the lines tested have the same tolerance level at seedling stage. When tested at vegetative and reproductive stages, the ultimate parameter in selecting saline tolerant lines is its yielding ability under saline condition. It is useful in selecting lines within as well as between populations/crosses.

Designation	Heading (d)	Grain yield (g pot ⁻¹)	Designation	Heading (d)	Grain yield (g pot ⁻¹)
IR10198-66-2	81	11.7	IR65192-4B-3-2	90	6.0
IR45427-2B-2-2B-1-1	90	6.2	IR65192-4B-4-2	90	12.9
IR5447-3B-10-2	88	9.5	IR65192-4B-14-1	85	0.0
IR58427-5B-15	88	8.7	IR65195-3B-4-2-1	84	7.3
IR58430-6B-14-1-2	85	4.0	IR65195-3B-6-3-2	90	1.2
IR59418-7B-21-3	85	12.3	IR65195-3B-9-2-1	90	1.5
IR60483-2B-17-2-1-2	90	13.2	IR65195-3B-13-2-3	90	6.9
IR60483-2B-18-3-3-3	90	5.0	IR65195-3B-19-1-1	85	1.7
IR61920-3B-7-2-2	88	11.1	IR65196-3B-5-2-2	90	7.1
IR61920-3B-8-1-1	88	5.3	IR65196-3B-13-3-2	90	9.8
IR61920-3B-15-2-2	88	9.6	IR65196-3B-10-2-3	90	0.2
IR61920-3B-18-2-2	88	5.9	IR67075-2B-2-2	81	18.8
IR61920-3B-19-3-2	88	18.6	IR67075-2B-5.2	81	6.5
IR61920-3B-24-2-2	88	6.9	IR67075-2B-15-1	85	2.3
IR63307-4B-4-3	90	3.4	IR67075-2B-18-2	81	6.9
IR63307-4B-9-2	90	12.8	IR67075-2B-20-2	81	8.1
IR63307-4B-13-2	90	7.1	IR67076-2B-6-1	81	5.2
IR63311-B-6-2-1-3	88	9.2	IR67076-2B-15-3	81	8.1
IR63311-B-3R-B-1-2	88	5.1	IR67076-2B-18-3	90	3.0
IR63731-1-1-4-3-2*	90	7.2	IR29**	90	0.7

Table 7.Seeding to heading duration and grain yield of advanced breedling lines
grown under saline soil condition from 21-d-old to maturity using the new
technique.

*Tolerant check **Su

**Susceptible check

Modifications

The technique can be modified to suit any specific requirements. There are no restrictions on the type of soil, fertilizer use, salinity level, compounds/chemicals used to obtain salinity, and time of salinization. Clay pots, though available with side holes, are not suitable. Plastic pots of larger size may be used but we have not tested sizes larger than 12 cm diameter. For the water bath, plastic trays of any size or dimensions can be used but with very large trays, visual scoring of plants is difficult. The effects of salinity on growth and reproduction could be estimated by growing test entries in both saline and nonsaline water baths.

CITED REFERENCES

- Akbar M, Yabuno Y. 1974. Breeding for saline-resistant varieties of rice. II. Comparative performance of some rice varieties to salinity during early developing stages. Jap. J. Breed. 25:176-181.
- Akbar M, Yabuno Y, Nakao S. 1972. Breeding for saline resistant varieties of rice. I. Variability for salt tolerance among some rice varieties. Jpn. J. Breed. 22:277-284
- Clarkson DT, Hanson JB. 1980. The material nutrition of higher plants. Annu. Rev. Plant Physiol. 31:239
- Devitt D, Jarreli WM, Stevens KL. 1981. Sodium-potassium ratios in soil solution and plant response under saline conditions. Soil Sci. Soc. Am. J. 45:80-86
- Gregorio GB, Senadhira D. 1993. Genetic analysis of salinity tolerance in rice (*Oryza sativa* L.) Theor. Appl. Gen. 86:333-338.
- [IRRI] International Rice Research Institute. 1967. Annual report for 1967. Manila (Philippines): International Rice Research Institute. 308 p.
- Iwaki S, Ota K, Ogo T. 1953. Studies on the salt injury in rice plant. IV. The effects on the growth, heading and ripening of rice plants under varying concentrations of sodium chloride [in Japanese, with English summary] Proc. Crop Sci. Jpn. 22:13-14.
- Kaddah MT, Lehman WF, Meek BD, Robinson FE. 1975. Salinity effects on rice after the boot stage. Agron. J. 67:436-439
- Korkor SA, Abdel-Aal RM. 1974. Effect of total salinity and type of salts on rice crop. Agric. Res. Rev. 52(5):73-78.
- Lee KS. 1995. Variability and genetics of salt tolerance in japonica rice (*Oryza sativa* L.). Ph D thesis, University of Philippines Los Baños, Laguna, Philippines.
- Maas EV, Hoffman GJ. 1977. Crop salt tolerance: current assessment. ASCE J. Irrig. Drainage Div. 103:115-134.
- Mori IK, Kinoshita T. 1987. Salt tolerance of rice callus clones. Rice Genet. Newsl, 4:112-113.
- Ota K, Yasue T. 1962. Studies on the salt injury to crops. XV. The effect of NaCl solution upon photosynthesis of paddy seed [in Japanese, with English summary]. Res. Bull. Fac. Agric. Gifu Univ. 16:1-16.

- Pearson GA, Ayers SD, Eberhard DL. 1966. Relative salt tolerance of rice during germination and early seedling development. Soil Sci. 102:151-156.
- Shimose N. 1963. Physiology of salt injury in crops. I. Effect of iso-osmotic pressure due to sodium chloride and sodium sulfate on the growth and absorption of minimal elements by rice plants. J. Sci. Soil Tokyo 34:107-111.
- Yoshida S, Forno DA, Cock JH, Gomez KA. 1976. Laboratory manual for physiological studies of rice. Manila (Philippines): International Rice Research Institute.