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A Rapid Method for Screening Rice Plants for Salt Tolerance

M.A.M Chowdhury and D.J.F. Bowling

Department of Plant and Soil Science
University of Aberdeen
Cruickshank Building, St Machar Drive
Aberdeen AB9 2UD, U.K.

ABSTRACT. *Nine varieties of rice (Oryza sativa L.), Sesuvium portulacastrum (a halophyte) and Phaseolus vulgaris (a glycophyte) were grown in culture solutions containing a range of concentrations of NaCl to evaluate the relationship between measured electrical potential differences (PD) and calculated driving forces, to screen the rice plant for salt tolerance. Growth of the plants and sodium concentrations of the roots were measured after 14 days. The PD between the external solution and the vacuoles of the outer cells of the root were also measured. This enabled the driving force on sodium at the cell membrences to be calculated using the Nernst equation. Sesuvium and those rice varieties that had previously shown salt tolerance, generated relatively more negative PDs and large driving forces when compared to salt sensitive plants tending to exclude sodium from the root. A linear relationship was observed between PD and driving force. This suggested that a simple measurement of PD for plants grown in a concentration of NaCl over a given period of time would provide a fairly rapid screening method for salt tolerance in rice and other species.*

INTRODUCTION

Rice (*Oryza sativa* L.) is a species native to swamps and freshwater marshes, and its cultivated varieties provide one of the world's most important food crops. However, salinization of some of the areas where it is grown has shown that it is very sensitive to salt. Salt concentrations as low as 50 mol/m^3 may be lethal even for most resistant varieties. Growth and grain yield are reduced in plants that survive in high salinity (Flowers and Yeo, 1981) indicating the necessity of developing rice varieties that are resistant to salinity. However, the physiological basis for salt resistance is not completely understood (Flowers and Yeo, 1981). Therefore, membrane PD and internal Na^+ concentration of a number of rice varieties which are known to be resistant or sensitive to salt were determined to find out a

screening technique for rice plants for salt tolerance. Membrane PD was compared with a salt sensitive plant bean *Phaseolus vulgaris* and a salt resistant *Sesuvium portolacastrum,* a mangrove swamps of East Africa.

MATERIALS AND METHODS

Rice seeds (*Oryza sativa* L.) from nine cultivars (*cvs* Pokkali (PK), BR 3, BR 8, DA 28, BR 20, Rajasail, Latisail, Kateribog and MI 48) were obtained from the Bangladesh Rice Research Institute. Dwarf bean (*Phaseolus vulgaris* L.) was obtained locally and *Sesuvium portolacastrum* (SP) a salt marsh plant was originally brought from Ghana and propagated vegetatively in the Department of Aberdeen University, U.K. Rice and bean seeds were pre-germinated and cuttings of *Sesuvium* were transplanted in plastic pots (diam. 15 cm) containing vermiculite at 28±1 °C and RH 85±5% under continuous light (250 $\mu E/m^2/s$) in a growth room. Seedlings were transferred into a culture solution in black painted glass jars (2.6 l) at 10-18 days after germination. They were grown under the same conditions as those of seedlings. Thirty six plants were grown from each plant type.

The culture solution contained KNO_3, $Ca(No_3)_2$, $MgSo_4$, KH_2PO_4, FeEDTA, at 0.83, 0.5, 0.25, 0.1, 0.05 mol/m^3, respectively and traces of B, Mn, Zn, Cu, and Mo as suggested by Jensen and Patterson (1984). *Sesuvium* and bean were aerated, but rice was left without aeration. Plants of SP, PK, BR 3 and MI 48 were grown at 0 (control), 25, 50, 100, 150 and 200 mol/m^3 of NaCl, whilst other cultivars (DA 28, BR 20, Rajasail, BR 8, Latisail, Kataribog and Dwarf bean) were grown only in 50 mol/m^3 of NaCl, and used for screening. The culture solutions were salinized after 7 days by adding a known weight of NaCl crystals to a fresh culture solution. The concentration was built up over 4 days by increments of 50 mol/m^3 to the desired levels (*viz.*, 25, 50, 100, 150 and 200 mol/m^3). The salinized culture solutions were changed at 7 and 14 days after planting, and the plants were harvested after 21 days. One cycle from germination to harvesting took 36-46 days. Plants were washed in tap water and divided into roots and shoots. After blotting with filter paper, samples were air dried for 5 min, and fresh weights were determined. Dry weights of roots and shoots were determined after subjecting to 65°C for 72 h. For determination of Na^+ in the root, 1 g of fresh material was boiled in distilled water (25 ml) for 20 minutes. The extract was filtered and the filtrate was made up to 100 ml with distilled water. Sodium in the extracts was determined by a flame photometer (Jenway). The concentration of Na^+ in the tissue was calculated assuming a root density of 1.

The electrical potential difference (PD) was measured as per the method of Graham and Bowling (1977). A jar containing the plant was positioned close to a microscope and one intact root was placed in a separate vessel under the microscope. Culture solution was circulated between the jar and the vessel prepared at 90 ml/h using a peristaltic pump (LKB). Micropipettes were filled from a 2.0 mm diameter self filling borosilicate glass capillary tubing (Clark Electromedical Instruments) on a microelectrode puller (Palmer) to give a tip of approximately 1 μm. They were filled with KCl (3000 mol/m^3) and connected to an electrode holder containing a Ag/AgCl electrode (WPI). The resulting microelectrode was connected to a high impedance voltage follower (WPI) which was in turn connected to a chart recorder (Bryans). The electrical circuit was completed with Ag/AgCl reference electrode (WPI mere 3).

The reference electrode was placed in the flowing solution in the vessel on the microscope stage, and the test microelectrode was mounted on a micromanipulator (Research Instruments), and inserted into the vacuole of the three outer layers of cortical cells of the root. Potential difference measurements were made on mature cells located approximately 10 mm from the root apex. Ten measurements were made on each root. During the measurements the plant was illuminated with a mercury vapour lamp giving 630 μE/m^2/s at leaf height. A period of about 1 h elapsed after the plant was installed before measurements began.

The driving force on sodium was calculated using the following equation:

$$\text{Driving force } (J \times 10^3 \text{ mol}^{-1}) = zF\,(E_m - E_n) \text{-------(1)}$$

where;

z = valency of the ion
F = Faraday (0.096 J mol^{-mv})
E^m = Transmembrane PD
E_n = Nernst potential for Na,
58/z log (Na outside)/(Na inside) at 20°C.

It was assumed that Na^+ was in equilibrium between the tissue and the external solution and that both the PD and the internal Na^+ concentration were applied to the vacuole.

RESULTS AND DISCUSSION

Figure 1 shows the effect of salinity on the dry weight of the plants. Graphs represent mean value of 4 plants from each variety. All rice varieties reached peak dry weight at 25 mol/m^3 NaCl in the external solution. As external salinity increased, dry weight declined steadily. In contrast, *Sesuvium* showed an increase in dry weight with increasing salinity. Pokkali had the highest dry weight in the first six weeks of growth.

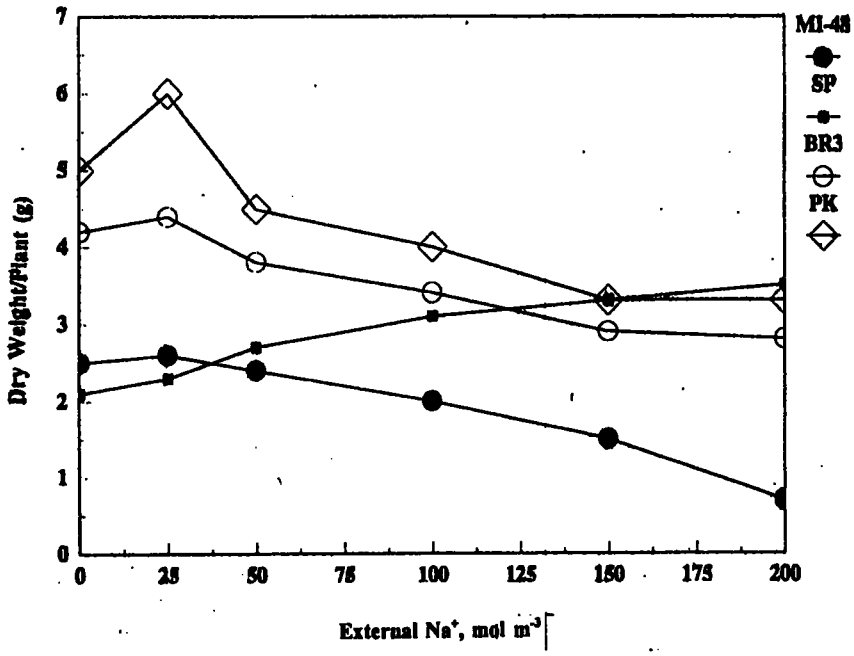

Figure 1. **Effect of Sodium Chloride on the growth (dry weight) of three varieties of rice (PK, BR 3 and MI 48) and *Sesuvium* (SP) after 40 days. (SEM ± 0.22).**

Figure 2 shows the accumulation of sodium by roots of the four plants during the same period. All four varieties showed a fairly steep increase in internal Na level as external Na increased, with MI 48 exhibiting a particularly sharp increase between 150 and 200 mol/m^3 NaCl.

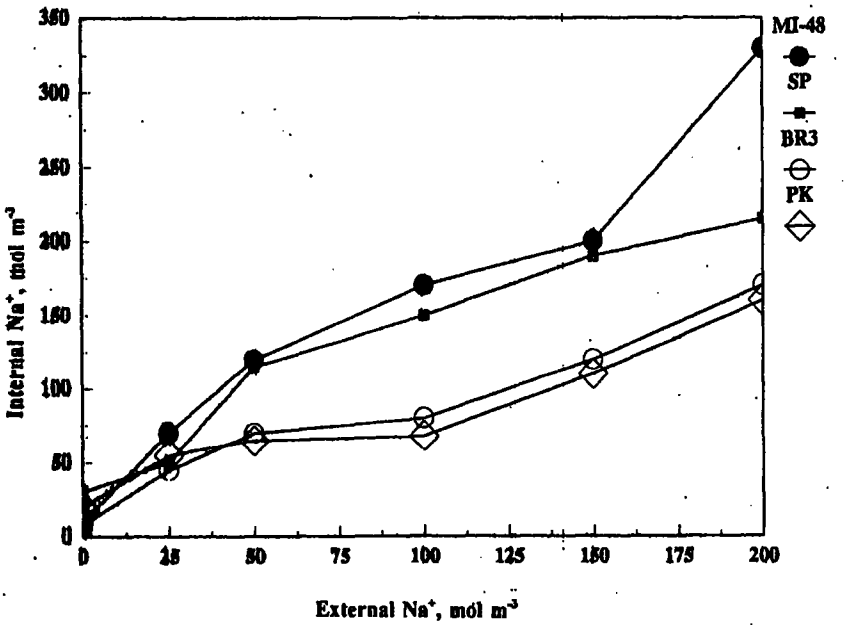

Figure 2. **Relationship between external Na^+ and internal Na^+ concentrations in the root for rice varieties PK, BR 3 and MI 48 and *Sesuvium* (SP). (SEM ± 2.86).**

In all plants, the transmembrane PD was depolarized, as the external NaCl concentration increased. The numerical value of the PD was approximately halved over the range of salinity used, *Sesuvium* generated the most negative PD ranging from -165 to -85 mV (Figure 3). There was a driving force tending to expel sodium from the roots in all the plants (Figure 4). It peaked at an external NaCl concentration of 25-100 mol/m^3. *Sesuvium* extruded Na^+ most vigorously, whilst that of MI 48 was the most feeble, generating only about 30% of the driving force exhibited by *Sesuvium*.

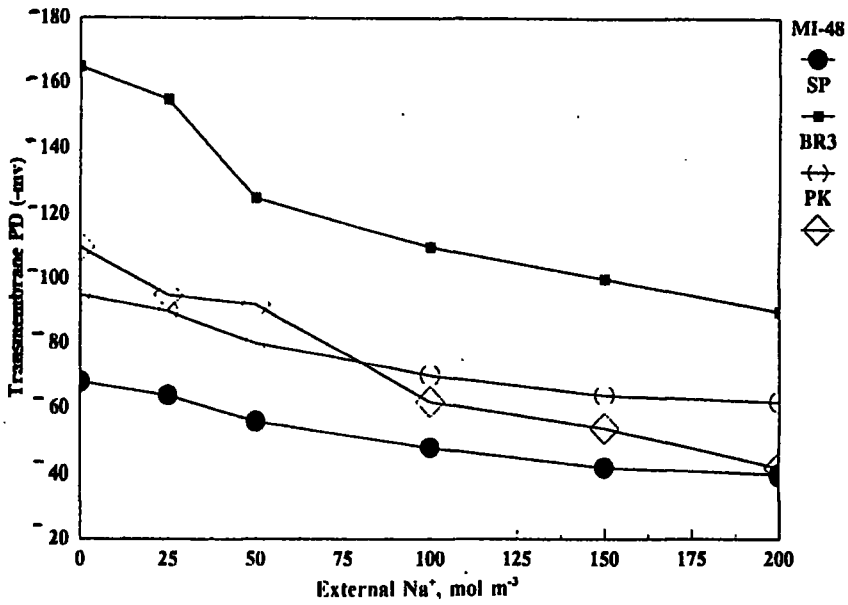

Figure 3. Relationship between Na^+ concentration and the $\overline{PD}$ of the outer cortical cells of the root of rice varieties PK, MI 48 and *Sesuvium* (SP). (maximum SEM ± 2.2 mV).

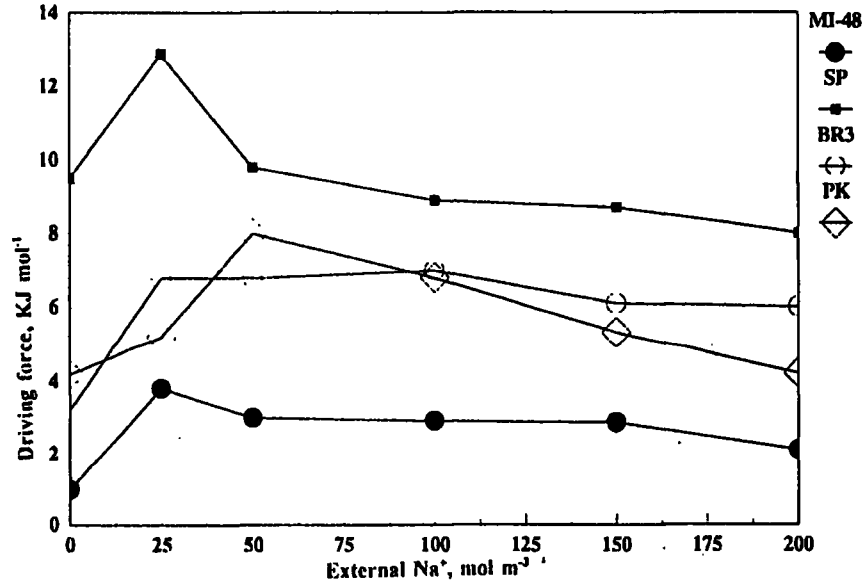

Figure 4. Driving forces on Na^+ calculated from the data in figures 2 and 3.

At first sight the driving force appeared to provide a means of screening the plants for salt tolerance as found in *Sesuvium*. This plant survived in sodium concentrations as high as 1000 mol/m^3 and had a greater ability to expel sodium than the three varieties of rice (Figure 4). As the driving force appeared to peak at 50 mol/m^3 Na^+, this concentration was chosen as an arbitrary screening level. When the roots from 40 day old plants of DA28, BR 20, Rajasail, BR 8, Latishail, Kataribog and Phaseolus were salinized with culture solution containing NaCl 50 mol/m^3, it was observed that the more negative the PD the greater the driving force (Table 1). The two parameters were plotted against each other from data obtained for all the varieties studied in this investigation. A curvilinear relationship was obtained as shown in Figure 5. *Sesuvium* had the largest driving force expelling sodium from the cells followed by rice variety Pokkali with most of the rice varieties investigated generating driving forces only about half that of Pokkali.

Table 1. **Transmembrane potential difference (PD), internal sodium concentration (Na^+), Nernst potential (E_n) and driving force (F) on sodium for roots from 40 day old plants salinised with culture solution containing NaCl (50 mol/m^3) for 11 days. (PD values are mean of 10 measurements ± SEM. Na values are mean from three plants ± SEM).**

Plant	PD (-mv)	Na (mol/m^3)	E_n (-mv)	F (kJ/mol)
DA 28	69.3±1.6	65.4±2.8	13.8	5.3
BR 20	67.0±4.7	63.4±6.6	20.3	4.5
Rajasail	65.6±1.3	71.7±4.1	20.4	4.3
BR 8	62.3±1.9	96.7±2.3	20.2	4.1
Latishail	61.2±2.5	65.0±4.1	20.9	3.9
Kataribog	58.3±1.9	81.6±1.5	23.0	3.4
Phaseolus	59.1±6.4	53.3±5.9	22.6	3.5

Rice is a highly salt-sensitive crop species (Yeo, *et al.*, 1986). In the present study the salt sensitivity of rice varieties was compared with those

of *Sesuvium portulacastrum* and a known salt sensitive plant, dwarf bean *Phaseolus vulgaris*. Thus, *Sesuvium* could be termed a halophyte, whilst all the other plants studied were glycophytes.

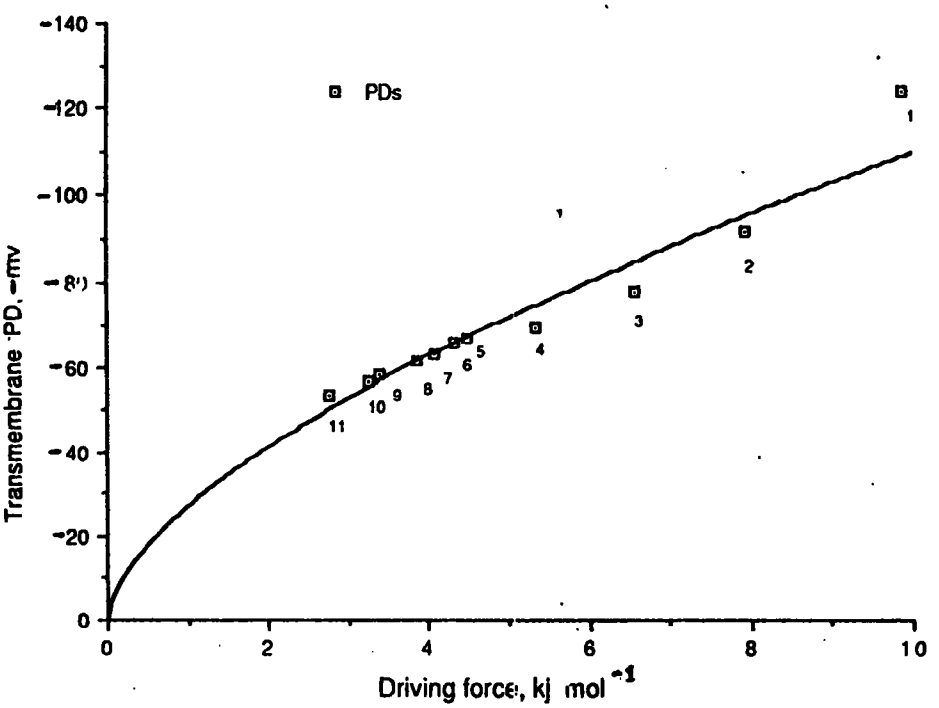

Figure 5. **Transmembrane PD plotted against driving force on sodium at 50 mol m^{-3} of NaCl.** 1. Sesuvium (SP), 2. Pokkali (PK), 3. BR3, 4. DA28, 5. BR20, 6. Rajasail, 7. BR3, 8. Latisail, 9. Kataribog, 10. MI-48, and 11. Dwarf bean.

All plant varieties accumulated sodium when treated with NaCl and low concentrations stimulated growth in the rice and bean, but growth declined at higher external salt concentrations. Sodium stimulated growth in the halophyte up to an external NaCl concentration of 200 mol/m^3. There was a negative PD in the outer cortical cells of all plants, which tended to become depolarized as the external NaCl concentration decreased (Figure 3). The PD in the *Sesuvium* was more negative than that in the rice varieties and the bean (Figure 4,5 and Table 1).

The driving force of sodium in most of the rice varieties was about 3-5 kJ/mole (Figure 5); but Pokkali, a traditional tall variety known to be relatively salt resistant, developed a driving force of 8 kJ/mol and BR 3, another High Yielding (HY) variety had a driving force of 7 kJ/mol. In contrast to all other plant varieties studied, the halophyte *Sesuvium* had a driving force of almost 10 kJ/mol.

Tolerance to salinity is not a simple process and a large number of physiological factors appear to affect salt resistance in rice (Yeo and Flowers, 1984). The majority of improvements to agricultural plants have been achieved by selection over extended periods of time. Selecting plants for salt tolerance, needs easily recognizable criteria which develop early in the life of the plant. These should be related closely to the fundamental mechanism of salt tolerance. We require physiological criteria which are easy to determine and are objective.

CONCLUSIONS

A number of criteria have been suggested to determine salt tolerance, (Yeo and Flowers, 1984) and our results demonstrated the ability of the root to exclude sodium as an alternative method. The results indicated that the driving force on sodium was closely related to the salt tolerance of the plants. We suggest that measurement of the driving force on sodium in plants grown in 50 mol/m^3 NaCl for two weeks as a suitable screening test. The method is simple and as PD and driving force are closely related, a simple measurement of PD would provide a rapid and objective assessment of a particular plant's ability to withstand salinity. We have not tested the method on a large number of plants of genetic diversity but the study suggests that it is possible to mount a single root under the microscope and obtain 10 measurements of PD from the same root tip in approximately 10 minutes. Therefore, a single operator can screen 50-100 plants a day.

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