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A method for screening rice plants for salt tolerance

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Abstract

A number of varieties of rice, a halophyte, *Sesuvium portulacastrum* and a glycophyte, *Phaseolus vulgaris* were grown in culture solution containing a range of concentrations of NaCl. Growth of the plants and internal sodium concentrations of the roots were measured after 14 days. The electrical potential difference (PD) between the external solution and the vacuole of the outer cells of the root was also measured. This enabled the driving force on sodium at the cell membranes to be calculated using the Nernst equation. It was found that *Sesuvium* and those varieties of rice that had previously shown salt tolerance generated relatively negative PDs and large driving forces tending to exclude sodium from the root. This suggested that a simple measurement of PD for plants grown in a given concentration of NaCl over a given period of time would provide a fairly rapid screening method for salt tolerance in rice and possibly other species also.

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 in the most resistant varieties. In plants that survive in high salinity growth and grain yield are reduced (Flowers and Yeo, 1981). Therefore there has been great interest in developing varieties of rice that are resistant to salinity. However, the physiological basis for salt resistance is not completely understood.

A significant finding is that plants which grow in high salinity (500 mol m^{-3} NaCl) such as *Suaeda maritima* accumulate sodium to very high levels (500 mol m^{-3}) in the vacuoles of the cells of the root and the shoot but keep the concentration of sodium in the cytoplasm down to about 100 mol m^{-3} (Yeo and Flowers, 1986). Above concentrations of about 150 mol m^{-3}, Na in the cytoplasm appears to be toxic, disrupting protein synthesis and enzyme activity. The osmotic potential of the cytoplasm is maintained by accumulation of organic compounds such as proline, betaine and sucrose (Flowers et al., 1977).

This response of the cytoplasm to high external salinity highlights the importance of the cell membrane. The plasmalemma of root cells is the part of the cytoplasm which first encounters the salt. The behaviour of this membrane in the face of large salt concentrations is therefore of great importance. There has been evidence for some time that there exists at the plasmalemma of most plant cells a sodium extrusion pump (MacRobbie and Dainty, 1958; Pitman and Saddler, 1967) which controls the level of sodium in the cytoplasm. It is possible that this control system is of such a distinctive structure in salt resistant plants to enable them to remove sodium from their cytoplasm much more efficiently than salt sensitive species.

Recently Xiaomu et al. (1993), reported that gene expression for H^+ ATPase in the membrane of roots of tobacco and *Atriplex* was stimulated more in the *Atriplex*, a salt resistant plant, than the tobacco, by NaCl. They found that mRNA for the H^+ ATPase accumulated more in the *Atriplex* than in the tobacco in the presence of NaCl. This suggested that NaCl was influencing the expression of a component of the mem-

brane transport system to a different degree in the two species. There is evidence that another characteristic of the cell membrane, the electrical potential difference (PD) is resistant to external sodium in *Atriplex* but not in the salt sensitive plant *Helianthus* where high external concentrations of NaCl produced large depolarisations of the PD (Cakirlar and Bowling, 1981; Anderson et al., 1977).

It was thought that the differences in membrane behaviour observed between species in the face of NaCl may also be seen, to a lesser extent, between varieties of the same species. It is relatively easy to determine the presence of a sodium efflux pump in roots by measuring the membrane PD and the internal sodium concentration. The driving force on sodium can then be calculated. In this paper we have done this for a number of different rice varieties, some known to be relatively resistant, and some sensitive to salt, and we have compared the results with those from a salt sensitive plant, the bean *Phaseolus vulgaris* and a salt resistant plant from the mangrove swamps of West Africa, *Sesuvium portulacastrum.*

Materials and methods

Seeds of rice (*Oryza sativa* L.) from 8 cultivars cvs Pokkali, BR3, DA28, BR20, Ragasail, Latibail, Kataribog and MI48 were obtained from the Bangladesh Rice Research Institute. Dwarf bean (*Phaseolus vulgaris* L.) was locally obtained and *Sesuvium portulacastrum* a salt marsh plant originally brought from Ghana had been propagated vegetatively in the Department. Rice and bean seeds were germinated in, and cuttings of *Sesuvium* transplanted into, Vermiculite in plastic pots (diam. 15 cm) at 28+/-1°C under continuous light (250 $\mu Em^{-2}\ s^{-1}$) in a growth room.

Seedlings were transferred to culture solution at 10–18 days old. They were grown in black painted glass jars (2.6 L) under the same conditions as those above. *Sesuvium* and bean were aerated but rice was left without aeration. The culture solution contained (mol m^{-3}); KNO_3, 0.83; Ca $(NO_3)_2$, 0.5; $MgSO_4$, 0.25; KH_2PO_4, 0.1; FeEDTA, 0.05 and traces of B, Mn, Zn, Cu and Mo. It was made up after Jensen and Pettersson (1984).

The plants were given NaCl after 7 days by adding a weighed amount of NaCl crystals to 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 m^{-3}). The salinized culture solutions were changed after 7 and 14 days and the plants harvested after 21 days. One cycle from germination to harvesting took 38–46 days.

Plants were washed in tap water and divided into root and shoot. After being blotted with filter paper the samples were air dried for 5 minutes before fresh weight was determined. For determination of dry weight, roots and shoots were put into separate paper bags and dried at 65°C in an oven 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 made up to 100 mL with distilled water. Na in the extracts was determined using a flame photometer (Jenway). The concentration of Na in the tissue was calculated assuming a root density of 1.

PD was measured according to the method used by Graham and Bowling (1977). A jar containing the plant was positioned close to the microscope and one root, still connected to the plant, was placed in a vessel on the microscope stage so that it could be viewed under the microscope. Culture solution was circulated between the jar and the vessel at 90 mL h^{-1} using a peristaltic pump (LKB).

Micropipettes were pulled from 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 a Ag/AgCl reference electrode (WPI mere 3).

The reference was placed in the flowing solution in the vessel on the microscope stage and the test microelectrode was mounted on a micromanipulator (Research Instruments). It was inserted into the vacuole of the outer three layers of cortical cells of the root. PD measurements were made on mature cells approximately 10 mm from the root apex. Usually 10 measurements were made on each root. During the measurements the plant was illuminated with a mercury vapour lamp giving 630 $\mu E\ m^{-2}\ s^{-1}$ at leaf height. A period of about 1 h elapsed after the plant was installed before measurements began.

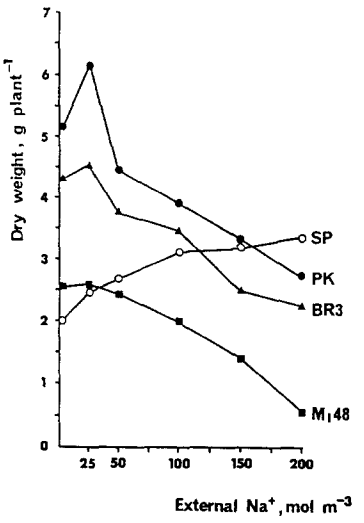

Fig. 1. Effect of sodium chloride on the growth (dry weight) of three varieties of rice PK, BR3 and MI48 and *Sesuvium* (SP) after 40 days. Points are means from 4 plants (maximum S.E.M +/−0.22).

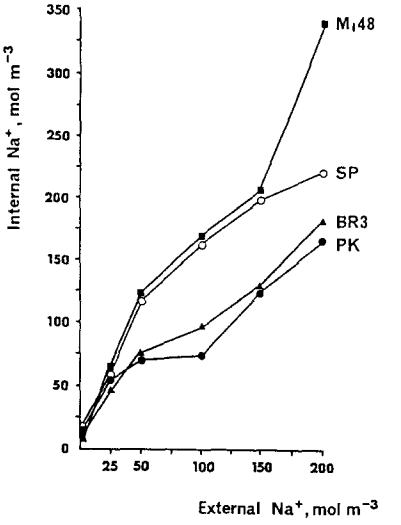

Fig. 2. Relationship between external Na^+ and internal Na^+ concentrations in the root for rice varieties PK, BR3 and MI48 and *Sesuvium* (SP). Data are means from 4 plants (maximum S.E.M. +/−2.86).

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

$$\text{Driving force } (Jx\ 10^3 mol^{-1}) = zF(Em - En) \quad (1)$$

Where z = valency of the ion, F = the Faraday (.096 J $mol^{-1}mV^{-1}$). Em is the transmembrane PD and En is the Nernst potential for Na. En = 58/z log. (Na outside)/(Na inside) at 20°C.

It was assumed that Na was in a steady state between the tissue and the external solution. It was also assumed that both the PD and the internal Na concentration applied to the vacuole.

Results

Growth and internal sodium concentration were studied over a wide range of external sodium concentrations in rice varieties Pokkali (PK), BR3 and MI48 and in *Sesuvium*. Figure 1 shows the effect of salinity on the growth of the plants as measured by dry weight. the rice varieties all showed a peak in dry weight production at 25 mol m^{-3} NaCl in the external solution and as external salinity increased dry weight declined steadily. In contrast, *Sesuvium* showed an increase in dry weight with increasing salinity. Pokkali put on the most dry weight in the six week growth period. Figure 2 shows the accumulation of sodium by the roots of the four plants during the same period. All four showed a fairly steep increase in internal Na level as external

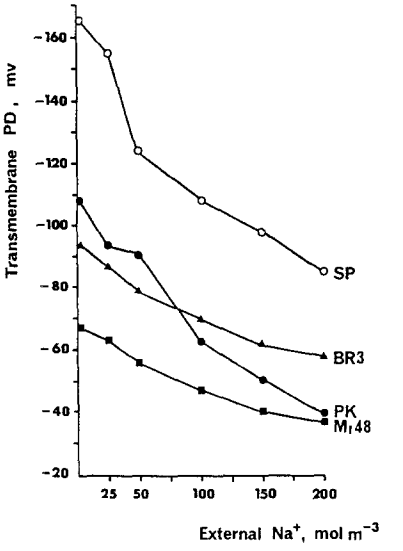

Fig. 3. Relationship between external Na^+ concentration and the PD of the outer cortical cells of the root of rice varieties PK, BR3 and MI48 and *Sesuvium* (SP). Data are means of measurements from four plants (maximum S.E.M. +/−2.2 mV). The negative signs on the ordinate denote the polarity of the vacuole.

Na increased with M48 exhibiting a particularly sharp increase between 150 and 200 mol m^{-3} NaCl.

The transmembrane PD was reduced as the external NaCl concentration increased, in all the plants. The numerical value of the PD was approximately halved over the range of salinity used. *Sesuvium* generated the

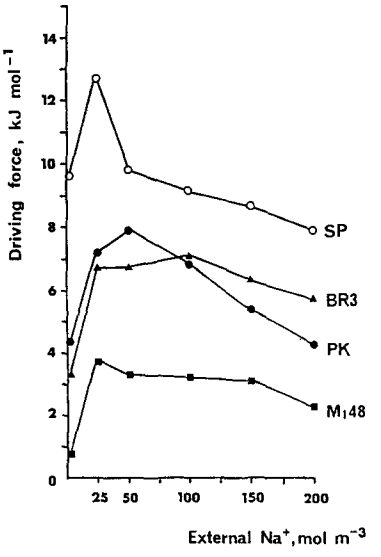

Fig. 4. Driving forces on Na^+ calculated from the data in Figures 2 and 3. (maximum S.E.M. +/−0.25 kJ mol^{-1}).

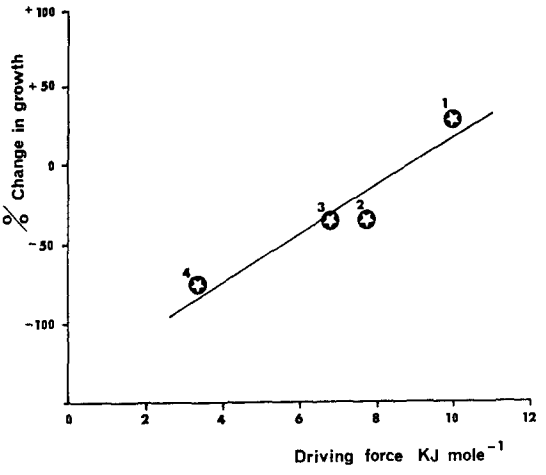

Fig. 5. Driving force at 50 mol m^{-3} NaCl plotted against % change in growth (dry weight) between 50 and 200 mol m^{-3} NaCl. Data taken from Figures 1 and 4. 1. *Sesuvium*, 2. Pokkali (PK), 3. BR3, 4. MI48. Maximum S.E.M. +/−0.25 kJ mol^{-1}.

most negative PD ranging from −165 to −85 mV (Fig. 3).

There was a pump expelling sodium from the roots in all the plants, as shown by the results in Figure 4. The departure from electrochemical equilibrium peaked at an external NaCl concentration of about 25–100 mol m^{-3}. *Sesuvium* showed the largest driving force whilst MI48 showed the smallest driving force on sodium, generating only about 30% of the driving force exhibited by *Sesuvium*.

At first sight the driving force appeared to provide a means of screening the plants for salt tolerance as we found that *Sesuvium*, which we observed could survive in sodium concentrations approaching 1000 mol m^{-3}, showed a much greater ability to get rid of sodium than the three varieties of rice with which it was compared in Figure 4. Therefore, as growth provides a general measure of salt tolerance we plotted driving force at 50 mol m^{-3} (Fig. 4) against % change of growth between 50 and 200 mol m^{-3} NaCl using data from Figure 1. The results are shown in Figure 5. A linear relationship was observed thus adding weight to the value of the driving force on sodium as a criterion for salt tolerance.

Therefore as the driving force appeared to peak at around 50 mol m^{-3} Na this concentration was chosen as an arbitrary screening level. Rice varieties DA 28, BR20, Rajashail, BR8, Latishail and Kataribog together with dwarf bean were grown for four weeks before being treated with 50 mol m^{-3} for a further two weeks. Sodium concentration in the root and cortical cell PD were then determined. The Nernst potential for Na and the driving force were calculated from the data and the results are shown in Table 1. It can be seen from Table 1 that there is a close relationship between driving force and PD. In fact when we plotted the two parameters from all the plants used in this investigation we observed a curvilinear relationship (result not shown).

Table 1. Transmembrane PD, internal sodium concentration, Nernst potential and driving force on sodium for roots from 40 day old plants salinised with culture solution containing NaCl (50 mol m^{-3}) for 14 days. PD results, mean of 10 measurements + SEM. Na results, mean from three plants + SEM

Plant	PD mV	Na mol m^{-3}	En mV	Driving force KJ mol^{-1}
DA28	69.3+1.6	65.4+2.8	13.8	5.3
BR20	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
BRB	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

Discussion

Rice is a very salt-sensitive crop species (Yeo et al., 1987) so we chose to compare our results with those from a salt resistant species, albeit a little studied one, *Sesuvium portulacastrum* and a known salt sensitive plant, dwarf bean *Phaseolus vulgaris*. Thus *Sesuvium* could be termed a halophyte whilst all the other plants we studied were glycophytes.

Driving forces on sodium were calculated from Equation 1 assuming that the sodium in the roots equated to the concentration in the vacuole and that the PD measured was that between the external solution and the vacuolar sap. Plants tend to accumulate sodium in the vacuole to higher levels than in the cytoplasm (Yeo and Flowers, 1986) and as the volume of the vacuole is much greater than that of the cytoplasm in fully expanded cells the total sodium content of the root will approximate to the sodium content of the vacuole. Experience has shown that on inserting a microelectrode into a cell, one cannot be certain whether the tip will penetrate the plasmalemma and the tonoplast to finish in the vacuole or merely pass through the outer membrane and remain in the cytoplasm. The evidence indicates that by far the larger component of the PD between the cell wall and the vacuole resides at the plasmalemma with only a small PD across the tonoplast. For example, Cakirlar and Bowling (1981) found that the PD across the plasmalemma of root cells of *Helianthus annuus* growing in 50 mol m^{-3} NaCl was 154 mV whilst the PD across the tonoplast was 18 mV. Therefore, the final position of the microelectrode tip was not a crucial factor in our measurements and very little error was introduced into our calculations by assuming that the PD we measured referred to the vacuole.

The calculations showed that there was a force tending to drive sodium out of the cells of all the plants investigated. The driving force on sodium in *Phaseolus* was 3.5 kJ $mole^{-1}$ (Table 1) and in most of the rice varieties it was about 3–5 kJ $mole^{-1}$ (Fig. 4 and Table 1) but Pokkali a traditional tall variety known to be relatively salt resistant developed a driving force of 8 kJ mol^{-1} and BR3 another traditional variety 7kJ mol^{-1} (Fig. 4). In contrast to all the other plants studied, the halophyte *Sesuvium* showed a driving force of almost 10 kJ mol^{-1}. Therefore, the known glycophyte showed about the lowest driving force and the halophyte the highest with the rice varieties arranged between them.

Tolerance to NaCl salinity is not a simple problem and a large number of physiological variables 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. To select plants for salt tolerance we need easily recognisable criteria which develop early in the life of the plant and which are related closely with the fundamental mechanism of salt tolerance. We require physiological criteria which are easy to determine and which are objective.

A number of criteria have been put forward already (Yeo and Flowers, 1984) and we would like to add the ability of the root to exclude sodium, as demonstrated by our results. The curve in Figure 5 indicates that the driving force on sodium is closely related to the salt tolerance of the plants. We would like to suggest that a suitable screening test would be the measurement of the driving force on sodium in plants grown in 50 mol m^{-3} NaCl for two weeks. The method is not very complicated and as PD and driving force are closely related (Table 1) a simple measurement of PD may be all that is required to provide a rapid and objective assessment of a particular plant's ability to withstand salinity. We have not tested the method on large numbers of plants but our experience suggests that it is possible to mount a single root under the microscope and take 10 measurements of PD in approximately 10 minutes. Therefore a single operator could screen 50–100 plants a day.

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