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Solution culture for screening rice varieties for sodicity tolerance

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SUMMARY

Sodic soils are widespread, especially in the Indo–Gangetic plain. Amelioration with gypsum is effective, especially when combined with growing a crop of rice. However, it has proved difficult to generate new varieties of sodic-tolerant rice, because of the difficulties of screening – other than in the field, where spatial variation of sodicity is notoriously high. Growing plants at high pH in solution culture in a controlled environment is problematic, because of the effects of pH on nutrient availability. This study evaluated a system of growing rice in a nutrient solution with added Tris but without minor nutrients, which were supplied in a foliar spray. Performance was evaluated from the change in weight and in appearance and correlated with performance in soil. Growing rice in the presence of Tris (4 mM, pH 8·6) provides a means of evaluating genotypic differences in response to sodicity.

INTRODUCTION

Salt-affected soils cover a significant fraction of the surface of the earth. Although chlorides commonly dominate such soils, there are large areas where carbonates and bicarbonates cause the soil to become distinctly alkaline (Ghassemi *et al*. 1995). Such sodic or alkali soils have pH values ranging from 8·5 to 10·5 and generally much higher than the values found in saline soils, where pH values are less than 8·8. The electrical conductivities of saturated soil pastes of sodic soils (<4 dS/m) are, however, lower than those of saline soils (>4 dS/m).

Sodic soils are particularly widespread in India, where they cover some 3·6 million ha (Singh 1998). The high pH values render these soils inhospitable to most plant species, and crops cannot be grown without some sort of amendment of the soil. In India, gypsum is commonly recommended at a rate of about 12–15 tonnes per hectare depending upon the Exchangeable Sodium Percentage (ESP) and texture of the soil. Although such applications may be effective, they are too costly for many farmers. There is also evidence that following treatment with gypsum, sodicity can increase over time where the underground water has residual alkalinity (Mehta 1994), increasing the long-term costs. Consequently, there is a demand for crop varieties tolerant to sodicity. This demand presents a formidable challenge to plant breeders due to the complex nature of salt-tolerance in plants and of its inheritance. For sodicity, the problems presented to physiologists are exacerbated by the practical difficulties of growing plants at high pH values.

In India, rice is a vital part of the process by which sodic soils are reclaimed for agricultural use. Reclamation commonly starts with treatment of soil with gypsum and then flooding of the fields in which a rice crop is planted. Although it is possible to reclaim sodic soils without gypsum treatment, the process takes longer than with gypsum (Datta *et al*. 1996). The choice of rice variety is critical because there are marked genotypic differences in sodicity tolerance. Conventionally, the selection of genotypes has depended on soil-based screening (Dargan *et al*. 1974; Srivastava *et al*. 1984; Qadar 1985, 1988; Sharma 1986; Singh *et al*. 1990). In the field, however, such screening is subject to the wide spatial variation characteristic of sodic soils and there is little or no control over environmental factors. Some control can be attained if experiments are carried out in lysimeters containing sodic soil. This has either to be transported from a nearby source or created artificially by

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adding $NaHCO_3$ to a normal soil. The latter takes many months before the pH stabilizes and even after a long equilibration time, there is always the possibility of toxicity from any excess soluble salt ($NaHCO_3$) that is not adsorbed at the exchange sites of the soil colloidal complex. This toxicity can create difficulty in separating adverse effects due to high pH from the presence of soluble $NaHCO_3$. Consequently, we have explored alternative solution-based screening.

The main aim of the study reported here was to develop a technique to mimic, in a solution culture, the sodicity of a sodic soil, in order to allow us to screen hundreds of rice genotypes for their tolerance. There are numerous solution-culture screening techniques for salinity tolerance but, as far as we are aware, no procedures that allow screening for tolerance to sodicity in solution culture. This is not surprising as micronutrients are precipitated from solution at the high pH values associated with the presence of HCO_3^- and CO_3^{2-} anions (Swarup 1982; Singh & Abrol 1985).

MATERIALS AND METHODS

A number of rice (*Oryza sativa* L.) varieties, obtained from the Central Soil Salinity Research Institute (CSSRI) or the International Rice Research Institute, were used for this study. The tolerance of CSR10, MI-48 and Bas370 to sodicity has already been established based on field experimentation over a number of years in sodic soils at CSSRI. CSR10 is more tolerant than Bas370, which is similar in tolerance to MI-48: they represent the extreme and the middle of the tolerance range seen in rice (Mishra 1994). A purple accession from IRRI was also investigated: because of its colour, this salt-sensitive genotype is regularly used in field experiments as a demarcation between genotypes. Plants were grown in a modified Yoshida culture solution (Yoshida *et al.* 1972) containing 1 mol/m^3 K^+ and 0·4 mol/m^3 Na^+ (Flowers & Yeo 1981) without micronutrients other than iron (and designated Yμ-); FeEDTA was replaced by FeEDDHA [ethylenediamine-di(*o*-hydroxy-phenylacetic acid) ferric sodium salt; MW 435 g; 34 μM] as it is the more robust iron-chelating agent at high pH. Other than Fe, the micronutrients $MnCl_2 . 4H_2O$, 9·4 μM; $(NH_4)_6 . Mo_7O_{24} . 4H_2O$, 0·075 μM; H_3BO_3, 18·8 μM; $ZnSO_4 . 7H_2O$, 0·15 μM; $CuSO_4 . 5H_2O$, 0·15 μM were supplied to the plants in the form of a daily foliar spray. Additional Fe (0·2% as $FeSO_4$) and Zn (0·1% as neutralized $ZnSO_4$) were also sprayed separately on a daily basis on to the leaves in order to satisfy the high demand of the plants for these two elements.

The experimental treatments were applied by varying the pH of the solution by the addition of 2–5 mM Tris (Sigma) buffer. As the Tris was likely to be biodegraded, the solution was changed every 4 to 5 days when the experiments were conducted during the winter or every 3 to 4 days in summer.

Plants were grown in 1-litre capacity black plastic beakers with perforated lids. For the first experiment, each of three holes contained two 10-day old rice seedlings (fixed with the help of cotton wool), allowing three genotypes to be planted in each beaker. There were three replicates of each treatment. In the second experiment, each variety was grown separately, to avoid any synergistic or antagonistic interactions between genotypes. Again, there were three replications of each treatment (six plants per beaker and three replicate beakers). The plants were grown either in a glasshouse at CSSRI or at the University of Sussex, UK. The glasshouses were maintained at 27–29 °C during the day and 25–27 °C at night. Supplementary light was supplied for 12 h per day at 400 μmol/m^2 per second PAR (measured with a Licor PAR sensor) from sodium lamps. The relative humidity ranged from 65 to 75%. Experiments were conducted between 1996 and 1999.

Shoot and root length, the number of tillers per plant and shoot and root fresh and dry weights were recorded after 2 weeks of Tris treatment. A sample of the dried plant material was extracted in hot acetic acid (0·1 M) and analysed for sodium and potassium using atomic absorption spectroscopy.

Plant growth in lysimeters

Ten rice genotypes, including those grown in solution culture, were planted in lysimeters at CSSRI, Karnal. These lysimeters were 6 m × 3 m in horizontal cross-section and 60 cm in depth. They were filled with a soil whose sodicity was created by adding $NaHCO_3$ to normal soil to produce three distinct pH_2 values (pH_2: pH of 1 part of soil and 2 parts of distilled water). Each rice genotype was grown (two plants per hill, in five rows of 3 m each with 15 × 15 cm spacing) at three pH_2 levels; viz. 9·2 (N.B. this pH is not stressful for rice in irrigated soil), 9·7 and 10·0. The middle three rows (of 2·7 m) of each genotype were harvested at the time of maturity.

X-ray microanalysis

Leaves collected for analysis were washed before drying to avoid any accumulation of remnants of the spray on the leaf surface. Part of the dried plant samples was ashed at a temperature of 550 °C in a muffle furnace. This ash was fixed, using double-sided sticky tape, to 'Wescor' filter discs on aluminium stubs and analysed for the concentrations of iron, zinc and manganese using a Leica Stereoscan 420 scanning electron microscope. The ashed samples were analysed for k_α counts using a current of between 500 and 7200 pA (about 20 keV energy) and a tilt of 70°; the

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detector distance was 18–19 mm and the magnification ×100. Counts were accumulated over 600–1000 s. The relationship between counts and the quantity of K was checked by determining the k_α counts for 3 μmol of K that had been ashed on a Wescor filter. A similar procedure was used with both 30 nmol and 3 μmol of Zn, Fe and Mn. The concentrations of the four elements were calculated as:

$$\text{K concentration} = (R \times V \times D)/d_{\text{wt}}$$

$$\text{Fe concentration} = (\text{Fe counts} \times \text{K conc.})/\text{K counts}$$

$$\text{Zn concentration} = (\text{Zn counts} \times \text{K conc.})/\text{K counts}$$

$$\text{Mn concentration} = (\text{Mn counts} \times \text{K conc.})/\text{K counts},$$

where R is the K concentration from the atomic absorption spectrophotometer; V is the extraction volume in ml (30 ml); D is a dilution factor; d_{wt} the dry weight of the sample and counts $= k_\alpha$ counts for each element.

RESULTS

The change in pH that can be brought about by addition of sodium hydroxide or sodium carbonate to Yoshida culture solution is not stable, even in the absence of plants. The pH values were in the range of about 1–2 units over a 24-h period, making the use of the hydroxide or carbonate anions impractical as a basis for screening for tolerance to high pH. Neither can bicarbonate solutions be used, as the pH cannot be raised above 8·25, too low a pH for screening for tolerance to sodicity. Consequently, the use of Tris buffer was explored.

Adjustment of the pH with varied concentrations of Tris buffer (Sigma, 2–5 mM, pH 7–9) resulted in a pH that was stable over a few days in the absence of any rice plants (data not presented). Fixed concentrations of Tris were used to adjust the pH to avoid the addition of any counter ions; low concentrations were used to avoid possible toxicity.

Is Tris buffer toxic to plants?

In the first series of experiments, 8-day-old rice seedlings of three varieties (CSR10, tolerant; MI-48, sensitive; and Purple, sensitive) were transplanted into a single beaker containing modified Yoshida solution (Yμ- with sprayed micronutrients), buffered with 2, 5 or 10 mM Tris. Thus the three varieties shared the same solution culture environment. The pH of the solution was neutralized (pH 7) with nitric acid (1 N) in order to eliminate the effect of differences of pH from any effect of Tris concentration per se. All the rice plants grew well at each Tris concentration, their growth remaining unaffected by the presence of Tris. None of the rice genotypes exhibited any deficiency or toxicity symptoms even after 8 days of growth in 10 mM Tris. This experiment suggested that Tris is not toxic to rice plants, at least over a period of 1 week: any effects at high pH in Tris should be attributable to the pH per se.

Growth of plants at various Tris concentrations/pH values

One-week-old rice plants (CSR10, MI-48 and Bas370) were again transplanted to the Yμ- culture solution containing Tris buffer: the pH of the solutions at the different concentrations of Tris varied from 8·5 (2 mM), through 8·7 (3 mM) and 8·8 (4 mM) to 8·9 (5 mM). During the course of the experiments, the pH decreased toward neutrality: the lower the concentration of Tris and the older the plants, the faster the drop (data not presented). Since the pH of the buffered Yμ- is stable over time in the absence of plants, this drop in pH was attributed to net proton extrusion from the roots.

The visual appearance of both shoots and roots clearly differed among varieties that differed in tolerance. Leaves of the sodic-tolerant variety CSR10 were lush green even after 1 week in pH 8·8 (4 mM Tris). Older leaves of the sodic-sensitive MI-48 were severely damaged at a pH as low as 8·5 (2 mM Tris). The first symptoms of damage to a plant from the high pH were yellowing of the oldest leaf, followed by its death and complete browning. Other leaves showed similar symptoms, worsening in sequence from younger to older. This trend was seen in all three varieties: the yellowing and browning started from the tip of the leaf and spread toward the base. The older (1st and 2nd) leaves of Bas370 showed signs of damage at pH 8·5 by the 3rd day of the Tris treatment. This damage was less severe than in MI-48 but certainly worse than in CSR10. At pH levels above 8·8 (5 mM Tris), even the third and fourth leaves of MI-48 and Bas370 were completely dead after 5 days (the effect of Tris at this higher concentration of 5 mM was quite severe and not recorded after this time). The leaf damage resulted in reduced growth: there was less reduction of the growth of the more tolerant CSR10 (23.9%; in 3 mM Tris compared with growth in 2 mM Tris) than that of the more sensitive Bas370 (38.9%) and MI-48 (31.3%). The rooting pattern was also affected by the pH of the solution. The roots of Bas370 were heavily branched at high pH, giving them a 'bushy' appearance. The roots of CSR10 and MI-48 did not branch to give this bushy appearance, but were of reduced length under high pH when compared with plants growing under control conditions (data not shown).

A second series of experiments was conducted in which genotypes were grown separately in individual beakers under various concentrations of Tris buffer (with three replications) in order to exclude the possibility of beneficial or harmful interactions between the roots of the tolerant and sensitive genotypes.

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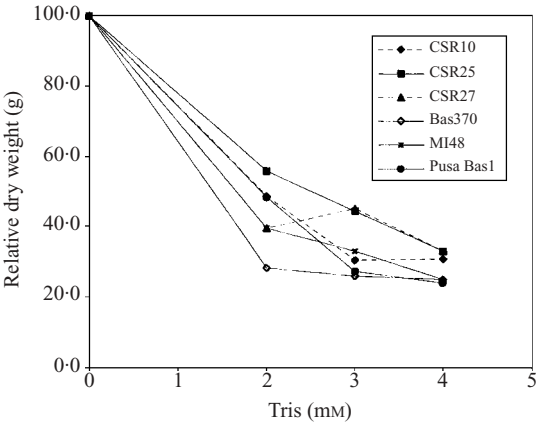


Fig. 1. The effect of increasing Tris concentration (and pH) on the growth (dry weight) of six genotypes of rice (*Oryza sativa*) expressed relative to that in the absence of Tris (Expt 2).

Growth was reduced at all elevated pH values, but with clear differences between the genotypes. The relationship between dry weight and Tris concentration (Fig. 1 and Table 1) suggested that difference would be best ranked in the presence of 2 mM Tris: differences are clear between the tolerant and sensitive genotypes (Table 2).

The major damage caused by high pH was visible on the second leaf, providing the possibility of a visual screening criterion: a diagrammatic representation of damage and score is shown in Fig. 2. The extent of the pH-affected area of the leaf was judged on a scale of 1–9 (a scale commonly used for scoring at the International Rice Research Institute), with 1 as the least affected leaf and 9 as the most severely affected (Fig. 2). Scoring leaf damage either after five days in 4 mM Tris or 10 days in 2 mM Tris placed the genotypes in a similar rank order as the effects of 4 mM Tris after 10 days on dry weight (Table 2). Scoring leaf damage after just 4 days allowed tolerant (CSR11) and sensitive (MI-48, Bas370 and Pusa Bas1) to be separated.

Micronutrient contents of leaves

The possibility that the deleterious effects of the high pH solutions on plant growth might be due to a lack of micronutrients (although supplied by foliar spray) was assessed by visual performance and investigated by analysis of ashed samples. The deleterious effects observed did not show any characteristics of those described for the deficiency of Fe (yellowing of the youngest leaf) or Zn (blanching of the leaves, IRRI 1983). X-ray analysis revealed that foliar spraying generally produced increasing concentrations of Fe, Zn and Mn in the leaves with increasing Tris concentration (from 4 to 20 μmol per gram dry weight; Table 3).

Plant growth in lysimeters

Ten genotypes of rice, including those used for solution culture, were grown in lysimeters at three distinct pH values: pH_2 values of 9·2 (non-stress for rice), 9·7 and 10·0. Only highly tolerant rice varieties such as CSR11 and CSR10 survived to maturity at a pH_2 of 10·0. At a moderate stress level (pH_2 9·7), the optimum for the discrimination among genotypes, sensitive and moderately sensitive types yielded poorly (Table 4).

DISCUSSION

Sodicity is a significant problem for agriculture in some parts of the world and yet is a difficult condition to mimic in the laboratory. The addition of bicarbonate ions to the culture solution commonly used for growing rice (Yoshida *et al.* 1972) did not result in a stable pH. Consequently, we added a buffer (Tris) to adjust the pH. This buffer was not itself toxic to plants at neutral pH. In order to avoid problems of precipitation of micronutrients, we omitted these from the solution and supplied the same elements in a foliar spray. Plants grown in this way did not show any of the common signs of nutrient deficiency and there was no significant difference in the response of plants of differing tolerance to pH to the absence of nutrients from their culture solution. The average concentrations of Fe, Zn and Mn in the plant (10, 10 and 6·5 μmol/g dry weight, respectively; Table 3) and the minimum concentrations measured (4, 3 and 3 μmol/g dry weight) were higher than those reported to be critical for deficiency in the shoots of rice, 1·25, 0·15 and 0·36 μmol/g dry weight, respectively (Yoshida 1981), suggesting that micronutrients were adequately supplied by spraying. The general increase in the concentration of Fe, Zn and Mn seen with increasing concentration of Tris (Table 3) probably reflects the decreasing growth, as quantities per plant were generally less affected by the treatment. On average, growth was reduced by a little over 70% when plants were grown in the absence of micronutrients and sodicity and compared with plants growing at the same pH (5·5) in normal culture solution containing micronutrients (data not presented). It is concluded that the deleterious effects on the plants due to high pH (Tris concentration) were not due to the non-availability of micronutrients. It is also evident from the results of x-ray analyses, that the accumulation of Fe, Zn and Mn was greater in the sensitive genotypes (Bas370 and M1-48) than the tolerant genotype (CSR10). This may be an indication of distinction between Fe and Zn efficient and non-efficient genotypes: the efficient genotypes are those that grow well under the minimal availability of these elements.

Although any effect of the treatment with Tris on the supply of micronutrients was countered by

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Table 1. *The effect of Tris (2 mM) on the dry weights (mg) of seven genotypes of rice* (Oryza sativa) (*Expt 2*)

Genotype	CSR10	CSR25	CSR27	Bas370	MI-48	Pusa Bas1
No Tris	224±16	342±45	291±58	203±40	248±39	237±34
2 mM Tris	109±9	191±21	115±9	57±15	98±9	114±27

Table 2. *Reduction in dry weight of 10 genotypes of rice* (Oryza sativa)*, expressed relative to the growth in Yoshida solution in the absence of Tris, and the extent of leaf damage, expressed as damage index* (*Fig. 2*) *after exposure to 2 mM Tris for 10 days or 4 mM Tris for 5 days*

	Relative reduction in dry weight in 2 mM Tris after 10 days	Leaf damage score	
		In 2 mM Tris after 10 days	In 4 mM Tris after 5 days
Pusa Bas1	51·8	7	9
MI-48	60·5	7	9
CSR27	60·4	3	5
CSR25	44·3	3	5
CSR23	43·1	3	5
CSR21	60·8	5	5
CSR20	39·0	3	5
CSR11	34·1	1	3
CSR10	51·4	3	5
Bas370	71·7	7	9

Table 3. *The effect of the concentration of Tris on the concentration and content of iron, zinc and manganese in the shoots of three genotypes of rice, differing in their tolerance of sodicity. Plants were grown for 21 days and the elemental composition determined by x-ray microanalysis after dry ashing*

Variety	Tris (mM)	Dry weight (mg)	Fe (μmol/g dwt)	Fe (μmol/plant)	Zn (μmol/g dwt)	Zn (μmol/plant)	Mn (μmol/g dwt)	Mn (μmol/plant)
CSR10	2	530	4·12	2·18	2·76	1·46	2·65	1·40
	3	358	7·85	2·81	6·34	2·27	5·20	1·86
	4	218	5·28	1·15	3·41	0·74	3·67	0·80
	5	141	10·54	1·49	8·88	1·26	6·59	0·93
Bas370	2	561	6·10	3·42	7·48	4·20	4·34	2·44
	3	516	8·69	4·49	10·9	5·63	5·89	3·04
	4	461	10·24	4·74	13·01	6·03	10·83	5·02
	5	223	17·75	3·96	15·66	3·49	8·84	1·97
M1-48	2	540	12·09	6·53	14·79	7·99	5·46	2·95
	3	648	6·56	4·25	9·54	6·18	7·67	4·97
	4	384	11·53	4·42	10·09	3·87	8·12	3·11
	5	268	20·48	5·49	21·64	5·80	9·15	2·45

spraying the leaves, the change of pH had a dramatic effect on the concentration of phosphate in solution as calculated by the program MinteqA2 (MINTEQA2 1991). Inorganic phosphate, which was supplied at a concentration of 0·48 mM, was reduced to 0·25 μM at pH 8·5. In spite of the low concentration of P in solution, plants of sodic-resistant lines still grew. Rice is capable of reducing the P concentration in solution to vanishingly small concentrations (R. Chhabra, personal communication) and at high pH any precipitated P will presumably continue to equilibrate with that in solution, providing an effective reservoir. Additionally, plants of the age used in these experiments will mobilize P from reserves in the

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Table 4. *The yield performance (and percentage reduction in yield) of 10 rice genotypes at varying sodic stresses generated in soil in lysimeters (and characterized by the value of pH_2: see Materials and Methods)*

	Grain yield (t/ha)			Reduction (%)	
	pH_2 9·2 (normal)	pH_2 9·7	pH_2 10·0	pH_2 9·7 over normal	pH_2 10·0 over normal
CSR11	5·8	4·1	2·2	29·3	62·1
CSR10	5·5	3·6	1·9	34·5	65·5
CSR23	6·3	3·9	1·2	38·1	81·0
CSR20	6·2	3·8	1·3	38·7	79·0
CSR27	5·9	3·6	1·3	39·0	78·0
CSR25	6·0	3·5	1·2	41·7	80·0
CSR21	5·7	3·1	0·9	45·6	84·2
Bas370	2·6	1·4	0·0	46·2	100·0
Pusa Bas1	4·3	1·9	0·0	55·8	100·0
MI-48	5·4	2·1	0·0	61·1	100·0

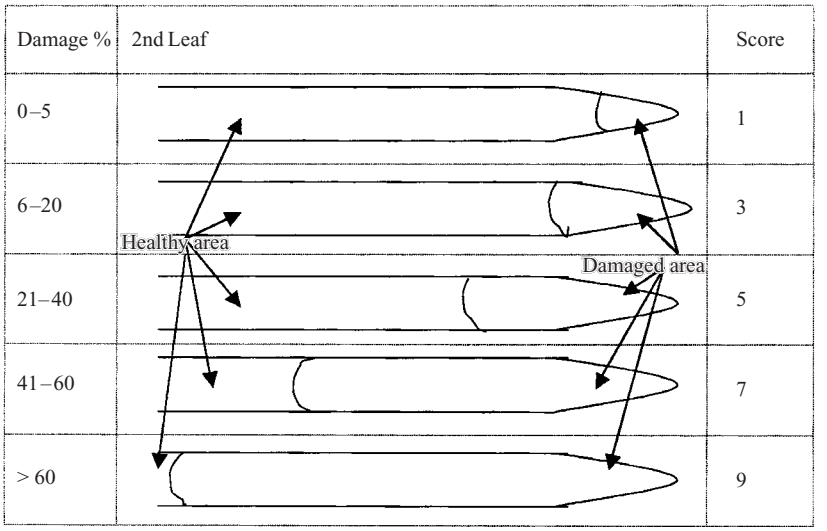


Fig. 2. An index to represent the effect of different concentrations of Tris (and hence pH values of the solution) on the proportion of healthy and damaged tissue in the second leaf of rice.

seed. Rice lines do vary in the efficiency with which they accumulate and utilize P (Fageria *et al.* 1988; Aslam *et al.* 1996) and fertilization with P does alleviate the effects of sodicity on rice, with a particularly large effect on sensitive genotypes (Qadar 1998). Growth of rice is stimulated by P concentrations above about 1 μM (Aslam *et al.* 1996), although at higher concentrations P is toxic in the presence of salinity. Since our aim was to separate sensitive and tolerant genotypes, the low P in the screen represents a positive rather than a negative attribute.

A range of responses to growth in Tris was clear. These could be measured as a reduction in dry weight and assessed by scoring the condition of leaves. Scoring was possible after 4 days at a higher pH (8·8, 4 mM Tris) and produced the same rank order as scoring after 10 days at a lower pH (8·5, 2 mM Tris). The rank order correlated broadly with the reduction in dry weight, although for two genotypes (CSR27 and CSR21), the effects on dry weight were marginally greater than would be expected from the degree of damage seen on the leaves. Nevertheless, sensitive (Pusa Bas, M1-48 and Bas370) and resistant (CSR11) lines could be clearly distinguished.

The test of any laboratory-based screening procedure is its ability to predict results for the field. The rank order for the effects of Tris (4 mM) on dry weight was very similar to the effects of pH_2 on yield and was

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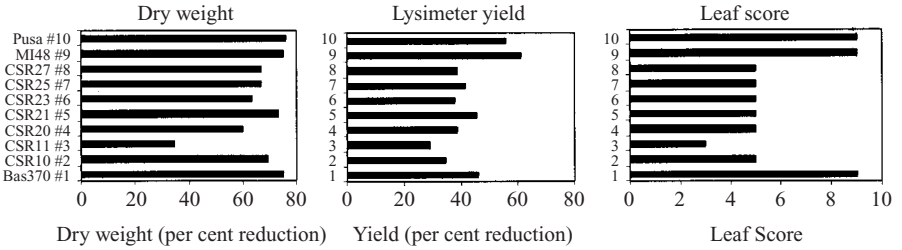


Fig. 3. Responses of 10 genotypes of rice to salinity. The ranking was based on the effects of Tris (4 mM) on per cent reduction in dry weight; pH_2 on per cent reduction in yield and leaf damage assessed after 5 days in 4 mM Tris.

reasonably well predicted by leaf damage assessed after 5 days in 4 mM Tris (see Fig. 3). Only CSR10 performed badly in terms of its dry weight when growing in Tris. Clearly visual screening of leaf damage of plants growing for just a few days in Tris buffer is a viable means to separate genotypes of rice sensitive and resistant to sodicity.

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