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Salt tolerance in the halophytic wild rice, *Porteresia coarctata* Tateoka

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SUMMARY

While members of the genus *Oryza* are very sensitive to salinity, salt concentrations as high as 20 % of that of seawater had no adverse effect on the growth of the tropical wild rice (*Porteresia coarctata* Tateoka) in experiments undertaken in a greenhouse in the UK. *P. coarctata* plants accumulated sodium and chloride ions in the leaves, but maintained a Na:K ratio as low as 0·7, even after 6 weeks of growth in 25 % artificial seawater (ASW) where the Na:K ratio was 34. This ability to maintain a high K:Na ratio in the leaves is in part a consequence of the secretion of ions from the leaves. The ratio of Na:K in the secreted salt (more than 5 for plants growing in 25 % ASW) is similar to that measured by X-ray microanalysis in the vacuoles of hairs found in folds of the adaxial surface of the leaf lamina, suggesting that the secretions emanate from these hairs. The salt secreted by the hairs is an important factor in the salt-balance of the leaves: the consequences of these findings for the transfer of salt-tolerance from this species into cultivated rice are discussed.

Key words: Salinity, halophyte, salt-glands, *Porteresia coarctata*, X-ray microanalysis.

INTRODUCTION

Extensive screening of *Oryza sativa* for resistance to salinity has led, slowly, to the realization that a source of real tolerance to salinity is unavailable within the cultivated germplasm. Consequently, we and others have considered the possibility of wide-crossing as a source of 'resistance genes'. The value of this technique in introducing tolerance to salinity to an otherwise salt-sensitive species has been demonstrated in a few cases, perhaps the best known being the incorporation of genes from *Lycopersicon cheesmanii* into the cultivated tomato, *Lycopersicon esculentum*, by Rush & Epstein (1981). More recently, hybridization between wheat and *Thinopyrum bessarabicum* (Gorham *et al.*, 1986) has demonstrated the prospective value of wide crossing for cereal crops. As far as rice is concerned, however, there is a dearth of potential donors for tolerance to salt.

In a recent comparison of the relative tolerance (measured as the survival of seedlings in a salinized hydroponic system) of seven wild species of rice and the two cultivated species, *O. sativa* and *O. glaberrima*, none of the wild species was found to be nearly as tolerant as the most resistant of the cultivated lines of *O. sativa* tested (Nona Bokra; Akbar, Jena & Seshu, 1987). Of the remaining ten or so species within the genus *Oryza* there is no information concerning their tolerance to salinity. There is, as far as we are aware, only one relative of *O. sativa*, that is clearly salt-tolerant, being a native of salt-marshes in S.E. Asia (around the coasts of the Bay of Bengal, for example). This species, *Porteresia coarctata* Tateoka (formerly a member of the genera *Oryza* and *Sclerophyllum*; cf. Clayton & Renvoize, 1986) is a perennial grass (and the only species within the genus). The genus *Porteresia* resembles *Oryza*, but differs in the nature of embryo and leaf anatomy (Clayton & Renvoize, 1986): it has an extensive rhizome system similar to that seen in species of the genus *Spartina* found in temperate salt marshes. Although it has not yet been successfully hybridized with *O. sativa* (probably since it is a tetraploid; Richharia & Roy, 1965), the mechanism of its tolerance to salinity is worthy of investigation, since modern methods of wide-crossing (e.g. Laurie & Bennett, 1988) might enable hybridization to be achieved.

In this paper we describe the response of *P. coarctata* to increasing external salinity and the

role of salt secretion from the leaves in its tolerance of salt.

MATERIALS AND METHODS

Plant material

Material of *Porteresia coarctata* Tateoka was obtained from the International Rice Research Institute in the Philippines in 1984. Rhizomes were divided at the nodes into small pieces approximately 5 cm long and grown in a potting compost irrigated with tap water for some three years. Subsequently, plants were divided vegetatively by taking cuttings consisting of rhizome, roots and culms (approx. 1 g fr.wt). These cuttings formed the basis of all the experimental material used: propagation by seed is difficult in this species as the seed are recalcitrant (seed with a water content of 30% and appearing partly dried on the plant germinate successfully, but once the water content has dropped to less than 10% the seed fail to germinate; see also Probert & Longley, 1989). For experimental purposes, the plants were grown in sand irrigated either with the culture solution defined by Yoshida *et al.* (1972) or a modified Hoagland's solution. The latter contained (in mM): KNO_3, 1·2; $NH_4H_2PO_4$, 0·2; $MgSO_4$, 0·4 and $CaCl_2$, 1·0, together with the micronutrients (in μM): H_3BO_3, 18·5; $MnCl_2$, 3·7; $ZnSO_4$, 0·3; $CuSO_4$, 0·13; $(NH_4)_6Mo_7O_{24}$, 0·14 and FeNaEDTA, 113. In early experiments, the plants were irrigated daily with the solution. In later experiments the pots were placed in plastic containers (430 × 740 × 180 mm deep) and irrigated automatically four times per day. The solution was pumped from a reservoir and flooded the pots, before draining back to the reservoir.

Experiments

In order to simulate saline conditions, either sodium chloride alone or the ions of an artificial seawater (ASW: Harvey, 1966) were added to the Yoshida's or Hoagland's solution. The ASW formulation was produced by adding the following salts (with the weights in grams of added salts per litre of solution for the equivalent of 100% seawater in parentheses): NaCl (23·48); $MgCl_2$ (4·98); Na_2SO_4 (3·91); $CaCl_2$ (1·10) and KCl (0·66).

Various experiments were conducted to investigate the effect of salts on the growth. In expt 1, six cuttings were grown per pot (130 mm in diameter, 120 mm deep) and irrigated with the Yoshida-based ASW for 28 days. The treatments consisted of various concentrations (0, 10, 20, 30, 40 and 50%) of ASW (4 pots per treatment). In the highest concentrations used (50% ASW) the major elements and their concentrations were (in mM): Na, 229; Mg, 26; Ca, 6; K, 5·6; Cl, 269 and SO_4, 14.

In expt 2, the growth and ion balance of plants grown in 25% ASW was investigated in detail. Cuttings (about 1 g fr. wt) were potted (one per pot; 130 mm in diameter, 120 mm deep) and grown in the automatic irrigation system: the conductivity before and after adding 25% ASW was 1·03 and 10·5 dS m^{-1}, respectively. Five plants were harvested at the time the treatments were imposed and an expanding leaf tagged in each of another five separate pots. This leaf was washed at intervals (see below). Harvests were made after 14 (8 plants), 25 (8 plants), and 39 (10 plants) days. Previously 'tagged' leaves were removed and washed (2 × 5 min) in 7 ml water (early harvests) or 0·5 M sorbitol (later harvests). These leaves were dried for analysis. The remaining plant was washed from the sand under running tap-water (containing approximately 2 mM Ca^{2+}) and quickly rinsed in tap water. The free space of the roots was then washed in sorbitol (250 mM) at room temperature. After the sorbitol wash of the roots, roots and shoots were dipped for a few seconds in distilled water (1000 ml; room temperature), blotted and weighed. This procedure was expected to remove about 70% of the ions on the surface of the leaves (see also below). The material from each of the harvests was dried, stored desiccated and eventually milled. Samples of the shoot, root and rhizome were extracted and analysed for Na, K and Cl (see below).

Two further experiments were performed to investigate the accumulation of ions in and on leaves following salinization. In expt 3, plants were irrigated with the modified Hoagland's solution containing either 0, 100 or 200 mM NaCl and the sodium that could be washed from a known length (between 130 and 220 mm) of the leaves with water (while the leaves were still attached to the plant) followed over 33 days from the time of adding salt. Two leaves were washed for plants growing in the absence of salt, and four leaves from the salinized plants. Washing consisted of immersion twice in 7 ml distilled water for 5 min followed by determination of the total amount of sodium in the two washings (see also below). In expt 4, four leaves from each of five plants were harvested prior to salinization, and 6 and 29 days after the addition of 25% ASW in modified Hoagland's solution. The ion concentrations in leaves of different ages (from leaf 1, the oldest to leaf 4, the youngest) on a single shoot were determined as was the amount of salt that could be washed from the leaves.

Finally, plants were grown to produce material for microscopy. Thus in expt 5 plants were grown for 4 weeks in Yoshida's solution alone or with the addition of 100 or 200 mM sodium chloride. For all the experiments, the plants were grown in a greenhouse.

The greenhouse was heated to a minimum of 25 °C by day and 20 °C by night. Upper temperatures were limited in summer by automatic venting to 28 ± 3 °C. Air was stirred by two roof-

mounted fans adjusted to give an average velocity of 0·5 m s^{-1} measured with a hot-wire anemometer (ELE International Ltd). The saturation water vapour pressure deficit varied between 0·3 and 3·0 kPa. Supplementary light was supplied for 12 h per day at 400 μmol m^{-2} s^{-1} photosynthetically active radiation (measured with a LiCor LI190 SB sensor) from high-pressure sodium lamps mounted in reflectors (GEC SON/T 400 W, Camplex Plantcare Ltd). Maximum photon fluence rates were those of the solar radiation at the time and were up to 1000 μmol m^{-2} s^{-1} for short periods. The inevitable variations in growth conditions with time of year included maximum photon fluence rate, daylength, daily maximum temperature, and humidity.

Measurement of ion contents

Ion contents were measured following extraction in acetic acid (0·1 M) by atomic absorption spectrophotometry (Pye Unicam SP9 800). Chloride was determined using a chloride electrode (EIL) in conjunction with an electrometer (Vibron). A complication to such measurements is the excretion of salt by the leaves, so that a significant proportion of the apparent ionic content of a leaf is external to and not within the cells. Consequently, it was necessary to develop and evaluate a procedure for washing the surface salt from the leaves (cf. Boon & Allaway, 1982).

In the first instance, leaves were detached and the efflux of ions from the leaf followed by transferring the leaf contained in a mesh-ended tube between successive samples of distilled water (20 ml). More than 70% of the ions lost in 5 min were lost within the first 40 s. Subsequently, leaves still attached to plants growing in the Yoshida culture solution containing 200 mM NaCl were washed by inserting them into test tubes containing water (7 ml). The water was changed after 1, 3, 5 and 10 min: washing for 3 min in this way removed 80–90% of the salt that could be removed in 10 min. Washing in sorbitol (0·5 M) rather than water made little difference to the removal of ions. The leaf is deeply ridged with a thick cuticle and is very resistant to wetting, presumably commensurate with tolerance to submergence in seawater. This may explain the lack of damage caused by osmotic shock. Nevertheless, sorbitol was used in the majority of cases. The procedure finally adopted involved two immersions for five minutes each in 7 ml of distilled water (early experiments) or 0·5 M sorbitol (later experiments), leaves being gently agitated during this period.

Microscopy

Leaves of plants grown in the presence (100 or 200 mM NaCl; expt 5) and absence of added sodium chloride were prepared for microscopy. Small pieces (3–5 mm) of tissue were cut from the apex, middle and base of the leaf blade and fixed in glutaraldehyde (5%) or 2 h, followed by OsO_4 for 2 h; in each case the fixatives were dissolved in the culture solution with which the plants had been irrigated – Yoshida's ±NaCl. Following dehydration through a graded series of ethanol (20 min each in 25, 50, 75, and 90% aq. ethanol and then two changes in absolute ethanol) and two changes of propylene oxide (20 min each), the tissue was embedded in Spurr's resin (Spurr, 1969). Thin sections (90–150 nm) were post-stained with uranyl acetate-lead citrate and the sections examined with a Jeol 100S electron microscope at 80 kV. Transverse sections were also cut (1–2 μm) from the same blocks for examination by light microscopy when stained with toluidine blue (in phosphate buffer, at pH 6·6).

The leaves were also examined using a scanning electron microscope (Jeol ASID 40 scanning attachment at 25 kV). Pieces of leaf were mounted on aluminium stubs with double-sided adhesive tape and coated with gold in a sputter coater.

For X-ray microanalysis, leaf segments (1–2 mm) were cut from the middle portion of the blades of leaves that had been grown in sand irrigated with the Yoshida's solution either with (100 or 200 mM NaCl) or without added salt for four weeks. The segments were gently cleaned with paper tissue to remove salt crystals from the surface of the leaf and prepared for freeze substitution in acetone as described by Harvey, Hall & Flowers (1976). Thin sections (100–150 nm) were cut dry on an LKB Ultratome and mounted on 400 mesh nickel grids. Specimens were coated with carbon under vacuum to prevent the absorption of moisture from the atmosphere and to provide stability under the electron beam. Grids were irradiated at 100 keV with electrons using a Jeol-Jem 2000 FX electron microscope in the STEM mode. X-ray spectra were collected for a 100 s live time at a fixed beam current and analysed using a model AN-10000 analysis system (Link Analytical Limited): a probe size of 20 nm was used as described by Hajibagheri & Flowers (1989). Ion concentration at the analysed sites was determined by comparison of R

(R = elemental peak–background/continuum)

with those obtained from the appropriate calibration standards (Harvey, Flowers & Kent, 1984).

RESULTS

Response to increasing salinity

Growth both in terms of fresh and dry weight was significantly reduced by salt concentrations greater than 30% ASW (Fig. 1); there was, however, no significant adverse effect of concentrations as high as 20% ASW on the dry weight of the plants.

Growth and ion relations in 25% ASW

Well-rooted cuttings were treated with 25% ASW in Hoagland's solution. Five plants were harvested at the time of imposing the treatments, then 8 plants after 14 and 25 days and 10 plants after 39 days. Growth, as measured by the total dry weight of the plant material per pot, increased over this period from 0·27 g to some 1·7 g with a mean relative growth rate between days 25 and 39 of 0·025 g g^{-1} day^{-1} ($\ln wt = -0{\cdot}432 + 0{\cdot}0247t$; $r^2 = 100\%$). After an initial (up to day 14) increase of the ratio of shoot:(root+rhizome) from 0·7 to 1·6, this ratio remained approximately constant over the remaining period of the experiment (such that there was about 1·4 g of tops per g of below ground material). Between the roots and the rhizomes, dry weight was distributed approximately evenly, and the ratio did not change significantly over the period of the experiment. Changes in the dry weights of the various parts of the plants are illustrated in Figure 2.

Ion concentrations (Na, K and Cl) were determined within the main parts of the plants (leaves, roots and rhizomes). The sodium concentration (expressed per unit dry weight) in the plant increased significantly following the imposition of the salinity treatment. Sodium concentrations in shoots, roots and rhizomes rose by 4·3, 10·4 and 6·4 times, respectively, between the first (before salinization) and the last harvest (Fig. 2). Chloride concentrations were high in the shoots before the imposition of the salinity treatment (due to the chloride in the culture solution) and rose to 1·3 times the initial value by the

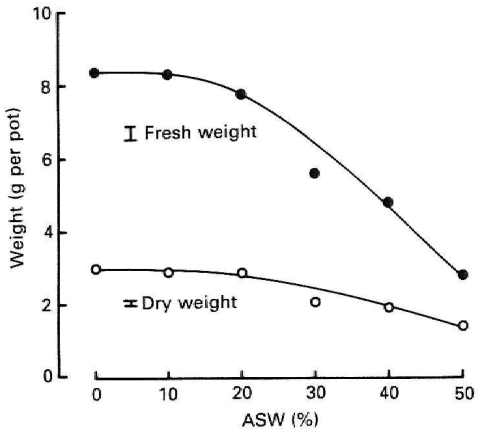


Figure 1. The effect of salinity on the growth of the halophytic grass *Porteresia coarctata*. Plants were grown for 28 days in sand irrigated with a culture solution (Yoshida) containing various concentrations of artificial seawater. During the period of this experiment the saturation vapour pressure deficit ranged between 0·6 and 1·6 kPa: other conditions were as described in the Materials and Methods. Open circles, dry weight; closed circles, fresh weight – each of six plants per pot; vertical bars, L.S.D. ($P = 0{\cdot}05$).

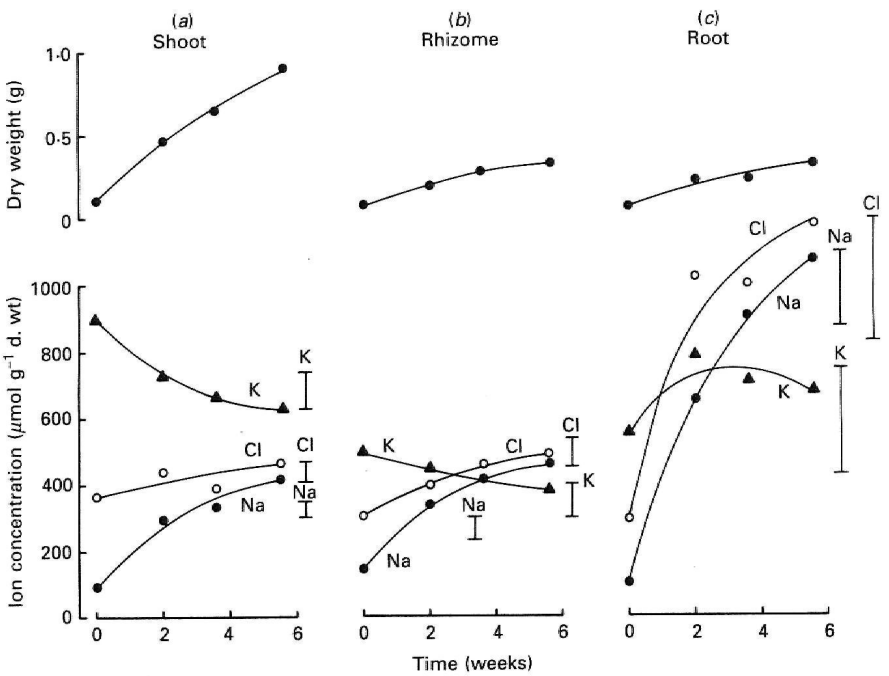


Figure 2. The change in dry weight and in ion concentrations in various parts of plants of *Porteresia coarctata* growing in modified Hoagland's solution containing 25% ASW. (*a*) Shoot, (*b*) rhizomes and (*c*) roots. The vertical bars represent the least significant difference ($P = 0{\cdot}05$) between the second and third harvests ($n = 8$): L.S.D.s are 14% larger for differences between the first ($n = 5$) and second harvests and 7% smaller for differences between the third and fourth ($n = 10$) harvests.

end of the experiment: the increases in the roots and rhizomes were 4·0 and 1·6 times respectively. Potassium concentrations fell significantly in the shoot, but did not change in the roots or rhizomes.

The picture of the change in ion concentrations in the shoot remains much the same if the concentrations are expressed on the basis of the water content, since the amount of water per unit dry weight is not significantly affected until the external concentration is above 50% ASW (from Fig. 1). Shoot water contents in plants grown in 25% ASW did not change significantly between days 15 and 39 (data not shown).

Because of the problems of washing whole shoots free of surface ions, individual leaves were harvested separately and washed: the sodium contents of the leaves and the washings were then calculated. Sodium concentration in these leaves rose with time, whether expressed per unit dry weight or per unit of leaf water (Table 1). Over the three harvests after salinization, the salt on the surface of the leaf (i.e. that in the washings from the leaf) averaged about 80% of that in the leaf itself, indicating that salt secretion is an important component of the salt balance in the leaf (data not shown).

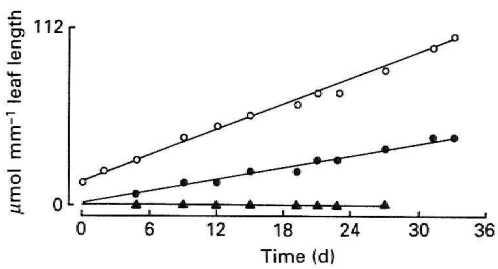
That salt secretion from leaves is sustained over a period of weeks and is a function of the external salt concentration was confirmed in a separate experiment (expt 3). Plants growing in the modified Hoagland's solution were salinized with either 100 or 200 mM sodium chloride and marked leaves washed on successive days. The sodium that could be washed from the leaves by water increased linearly with time and was greater the greater the external salt concentration (Fig. 3).

In a further experiment (expt 4), four leaves were harvested separately from each of five plants just prior to salinization with 25% ASW, 6 days later and then a further 29 days from adding salts. Over this period of time, there were no significant differences between the sodium, potassium or chloride ion concentrations (expressed per unit dry weight) between leaves of different age: mean leaf potassium concentration remained at $777 \pm 40{\cdot}4$ μmol g^{-1} d. wt. Sodium concentrations increased significantly from $160{\cdot}1 \pm 31{\cdot}5$ to $422 \pm \mu$mol g^{-1} d. wt in the first 6 days, but did not change significantly thereafter. The ion content of

Table 1. *Ion concentrations in the leaves of* Porteresia coarctata *following salinization with 25% ASW*

Time (days)	Ion concentration (μmol g^{-1} d. wt)		Ion concentration (mM)	
	Na	Cl	Na	Cl
0	24 ± 5	n.d.	n.d.	n.d.
4	234 ± 30	226 ± 19	128 ± 14	124 ± 19
25	318 ± 21	176 ± 28	192 ± 10	106 ± 17
39	489 ± 64	376 ± 73	234 ± 41	175 ± 56

Data are means $\pm$ standard error of the mean ($n = 5$ for $t = 0$; $n = 8$ for $t = 4$ and 25 and $n = 9$ for $t = 39$ days) for expt 2.



External NaCl (mM)	Slope μmol/mm/day	Intercept μmol mm^{-1}	r^2	No. of leaves
0	0·105	−0·60	89·5	2
100	1·38	−1·72	99·8	4
200	15·2	+2·56	99·6	4

Figure 3. The excretion of ions from leaves of plants of *Porteresia coarctata* growing either in culture solution (modified Hoagland's) alone or in culture solution plus 100 or 200 mM NaCl. The ions were washed, on successive days, into water from known lengths (130–220 mm) of marked leaves: these leaves remained attached to the plant throughout the period of the experiment.

Table 2. *The contents of and the amounts of sodium washed from leaves of the halophytic wild rice,* Porteresia coarctata *prior to (H1) and following (6 days, H2; and 29 days, H3; expt 4) salinization with 25% ASW in Hoagland's solution. Leaf 1 was the oldest on the shoot harvested and leaf 4 the youngest*

Leaf	Leaf content (μmol per leaf)			Leaf washings (μmol per leaf)		
	H1	H2	H3	H1	H2	H3
1	$0{\cdot}27 \pm 0{\cdot}12$	$1{\cdot}5 \pm 0{\cdot}48$	$1{\cdot}40 \pm 0{\cdot}62$	$0{\cdot}71 \pm 0{\cdot}31$	$2{\cdot}0 \pm 0{\cdot}82$	$3{\cdot}0 \pm 0{\cdot}58$
2	$0{\cdot}95 \pm 0{\cdot}42$	$1{\cdot}8 \pm 0{\cdot}29$	$5{\cdot}80 \pm 3{\cdot}10$	$1{\cdot}20 \pm 0{\cdot}47$	$2{\cdot}1 \pm 0{\cdot}59$	$5{\cdot}3 \pm 1{\cdot}30$
3	$2{\cdot}70 \pm 2{\cdot}10$	$3{\cdot}0 \pm 1{\cdot}30$	$11{\cdot}0 \pm 1{\cdot}70$	$1{\cdot}50 \pm 0{\cdot}40$	$2{\cdot}9 \pm 0{\cdot}62$	$6{\cdot}8 \pm 0{\cdot}53$
4	$0{\cdot}58 \pm 0{\cdot}31$	$3{\cdot}4 \pm 1{\cdot}40$	$7{\cdot}70 \pm 0{\cdot}74$	$1{\cdot}50 \pm 0{\cdot}47$	$2{\cdot}3 \pm 0{\cdot}36$	$4{\cdot}5 \pm 0{\cdot}63$

Table 3. *The change in contents of and the amounts of salt washed from leaves of the halophytic wild rice,* Porteresia coarctata *over a period of 29 days following salinization with 25 % ASW in Hoagland's solution. Leaf 1 was the oldest on the shoot harvested and leaf 4 the youngest*

	Change in (μmol per leaf per day)	
Leaf	Leaf content	Leaf washings
1	39	80
2	167	141
3	280	183
4	244	104

individual leaves, clearly reflects not only the ion concentration, but also the size of the leaf. It provides a basis, however, from which the proportion of ions excreted can be compared with those accumulated in the leaf. Total leaf contents of sodium increased following salinization for all the leaves (Table 2). The amount of sodium washed from the leaves also increased, the greatest increase occurring for leaf 3. When the change in the amount excreted is calculated as a proportion of that accumulated by the individual leaves over the 29 days of the experiment (assuming the changes are linear with time as suggested by the data of Fig. 3), leaf 3 both accumulates and excretes more salt than the other leaves (Table 2). It is particularly interesting, however, that excretion is generally in excess of 50 % of that accumulated by the leaves, confirming that salt excretion is an important component of the salt-balance of the leaves of *P. coarctata* (Table 3).

The quantity of ions washed from the leaves (in 0·25 M sorbitol) was rather variable between leaves, but the overall averages for the ratios between the quantity washed from the leaves and that in the leaves were 3·1, 0·29 and 3·5 for Na, K and Cl, respectively, at the final harvest: a very much higher Na:K ratio in the washings than in the leaves themselves. The difference between the Na:K ratios in the leaves and the washings was highly significant for the two harvests following salinization, and when the data for these two harvests were pooled, the Na:K ratios were 0·73 in the leaves and 5·2 in the washings.

Salt hairs

The mechanism by which ions are excreted from the leaves is not certain, but circumstantial evidence suggests that it is through the hairs on the leaves. These hairs, which are occasionally bifurcated (Figs 4 and 5), are present in folds on the adaxial surface of the leaf. They arise from the epidermis, appear to be unicellular with an electron-dense vacuole, and are

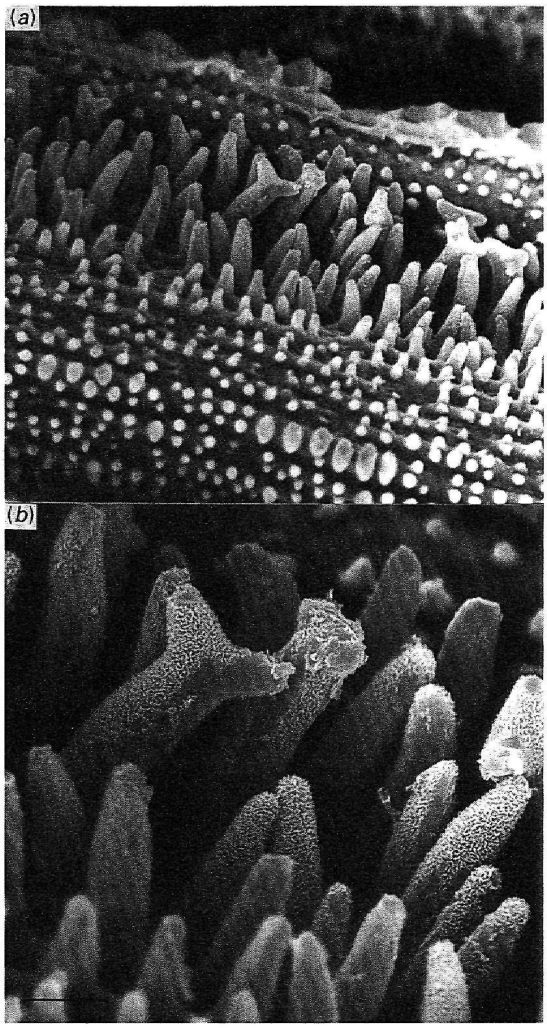


Figure 4. Scanning electron micrographs of the adaxial surface of a leaf of *Porteresia coarctata* grown for 4 weeks in Yoshida's culture solution with the addition of 200 mM sodium chloride, illustrating the numerous hairs (*a*). A few hairs are branched: they are covered in a cuticular wax and salt (*b*). The scale bar represents 8 μm in (*a*) and 5 μm in (*b*).

covered with cuticle (Fig. 6). As judged by preliminary counts of the numbers of hairs on mature leaves from plants that had been salinized for 4 weeks, salinization increased the number of hairs per cross section. X-ray microanalysis of hairs from plants grown either in the absence of salt or in the presence of 100 or 200 mM NaCl showed that there was an increase in the vacuolar concentrations of sodium and chloride within the hair cells with increasing external salt concentration (Table 4). The concentrations of these two ions were higher than in the mesophyll or neighbouring epidermal cells. In contrast, the potassium concentration in the hair was lower than in neighbouring cells (Table 4).

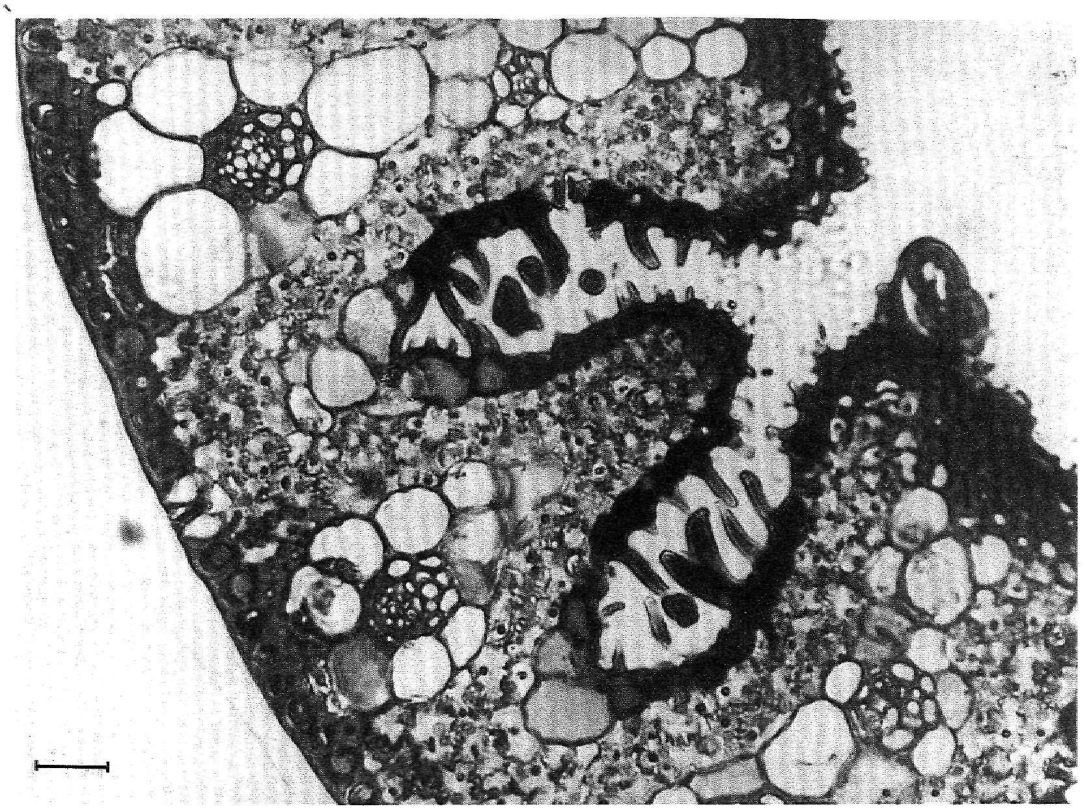


Figure 5. Transverse section of a leaf of *Porteresia coarctata* grown for 4 weeks in Yoshida's culture solution with the addition of 200 mM sodium chloride. Branched and non-branched hairs are clearly visible in furrows on the adaxial surface of the leaf. The scale bar represents 20 μm.

Table 4. *The ion concentrations within the vacuoles of cells from leaves of* Porteresia coarctata

Culture conditions (NaCl, mM)	Element	Elemental concentrations in various cell types (mmol l^{-1})		
		Epidermal	Mesophyll	Hair
0	Na	10±5	5±3	8±4
	K	80±28	66±16	6±3
	Cl	19±8	11±5	26±6
100	Na	110±42	79±26	255±39
	K	67±30	88±18	35±20
	Cl	100±23	128±37	203±55
200	Na	168±55	158±39	323±96
	K	70±43	50±22	58±19
	Cl	219±40	172±55	280±78

Ion concentrations, expressed as mmol l^{-1} analysed volume, were determined by X-ray microanalysis of thin sections prepared from freeze-substituted material. The figures represent the mean±the standard deviation of 4–6 separate determinations for each data point.

DISCUSSION

The plants used in this work had similar leaf anatomy to those described as *Oryza coarctata* Roxb. by Tateoka (1963), with large and small vascular bundles in the ribs of the leaves and small bundles under the furrows (Fig. 5). This arrangement is peculiar to *Porteresia coarctata* Tateoka (*Oryza coarctata* Roxb.) amongst the Oryzeae (Tateoka, 1963; Clayton & Renvoize, 1986) confirming the identification of the species used in these studies. Furthermore, the plants were clearly halophytic, a characteristic of *P. coarctata* (see Bal & Dutt, 1986).

Growth, in terms of dry weight, of *P. coarctata* was unaffected by salt concentrations up to 40–50 mM and plants survived in the concentrations to be found in 50% seawater. Growth, in the short term, was not found to be enhanced by salinity; this is a characteristic of the response of graminaceous halophytes to increasing salt concentrations (Greenway & Munns, 1980). In other reports, however, the growth of *P. coarctata* has been shown to be stimulated by salt (Bal & Dutt, 1986; Akbar & Seshu, personal communication); whether this is a consequence of the nature of the culture solution (not specified by Bal and Dutt) or the length of the period exposure to salt (much longer in the experiments of Bal and Dutt, and Akbar and Seshu than those reported here) remains to be determined.

Under saline conditions, the plants accumulated sodium and chloride ions, the highest concentrations

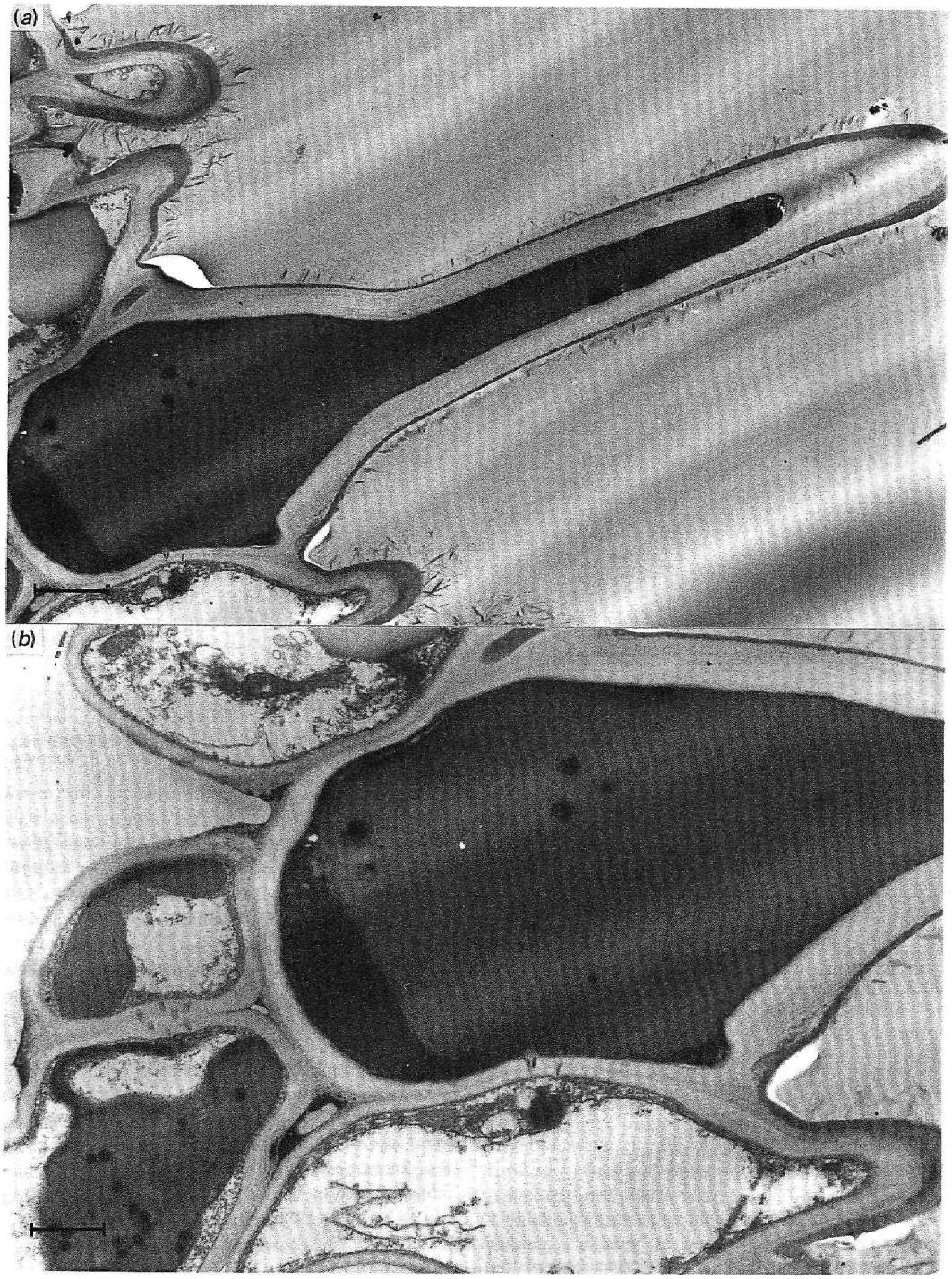


Figure 6. Transmission electron micrograph of a transverse section of a leaf of *Porteresia coarctata* grown for 4 weeks in Yoshida's culture solution with the addition of 200 mM sodium chloride, showing an epidermal hair from the adaxial surface. The hair lacks organelles, although plasmodesmatal connections can be seen between neighbouring epidermal cells. The scale bar represents 2 μm in (*a*) and 1 μm in (*b*).

being found in the roots. However, even after nearly 6 weeks in 25 % ASW (Na:K of 34), the Na:K ratio in the leaves remained at about 0·7, which is again characteristic of graminaceous halophytes (Flowers, Hajibagheri & Clipson, 1986). As far as *P. coarctata* is concerned, maintenance of this low Na:K ratio appears to be in part a function of high selectivity for sodium in the secretion of ions from the leaves.

The ability of *Porteresia coarctata* plants to excrete salts on to the surface of the leaf has been described previously and ascribed to the presence of 'special unicellular structures' found in the furrows on the adaxial surface of the leaf (Bal & Dutt, 1986). Bal and Dutt washed salts from the leaves with distilled water and found a Na:K ratio of between 3 and 4, when the plants were grown under saline conditions. In our experiments, the Na:K ratio in the washings from the leaves of plants grown in 25 % ASW was a little over 5, confirming the importance of secretion in maintaining the relatively low Na:K ratios in the leaves themselves.

The result of our analyses (by X-ray microanalysis) of the contents of the hairs on the leaves is powerful evidence in support of the contention (Bal & Dutt, 1986) that the secreted salt comes from the hairs. The Na:K ratio in the hairs of plants raised on NaCl was very much higher than that in the mesophyll cells, namely 7·3 in the hairs as opposed to 0·9 in the mesophyll for plants growing in 100 mM NaCl. These ratios may be compared with those recorded for the whole leaves – about 5·2 in the washings and 0·7 in the leaves – suggesting that the contents of the hairs give rise to the material that can be washed off the surface of the leaves.

The hairs are presumably salt-glands, as defined by Fahn (1988): '...specialized epidermal cells or trichomes, which play an active part in the secretion of solutions of mineral salts...'. Within the Poaceae, salt glands have been described in many species and from a number of tribes (e.g. Eragrostideae: Liphschitz & Waisel, 1974; Gorham, 1987; Cynodonteae: Liphschitz *et al.*, 1974; Skelding & Winterbotham, 1939; Levering & Thomson, 1971; Pappophoreae: Taleisnik & Anton, 1988). These glands, all of species within the sub-family Chloridoideae, are two-celled – with a basal cell and a cap cell (see Thomson, Faraday & Oross, 1988). The tribes Eragrostideae and Cynodonteae are closely related while there is thought to be a distant relationship between the Pappophoreae and the Eragrostideae (Clayton & Renvoize, 1986) and it is therefore perhaps not surprising that their salt-glands have a similar structure. The structure of the glands of *P. coarctata* are, however, even simpler than those described in the sub-family Chloridoideae, being unicellular: there is no indication of cap and basal cells. Whether they release ions simply by collapsing as do the bladder cells of *Atriplex* species (see Fahn, 1979) or whether a single gland is able to continue secretion is as yet not known. It is clear, however, that the salt secreted by the glands is a significant proportion of the ions arriving in the leaf (Table 4).

Gorham (1987) calculated that excretion from leaves of *Leptochloa fusca* was about 5 times the rate of accumulation in the leaves over a 24 h period, in contrast to the situation occurring in *Pappophorum philippianum*, where only 11 % of ions accumulating in the leaf are secreted (Talesnik & Anton, 1988). The situation in *P. coarctata* is somewhat similar to that in *L. fusca* and suggests that the possession of glands is of vital importance to the ability to withstand salinity. This together with the very low relative growth rate exhibited by these plants growing in 25 % ASW throws some doubt into the value of transferring genes from *Porteresia* to *Oryza sativa* as a means of increasing the salt-tolerance of rice. It may be possible to transfer those genes that produce salt-secreting hairs, but the background of a low growth rate would not be acceptable in a crop species.

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