Responses of the mangrove *Rhizophora mucronata* to high salinities and low osmotic potentials

G. Naidoo

Department of Botany, University of Durban-Westville, Durban

The effect of solutions of seawater or non-saline polyethylene glycol (PEG)-6000 on dry mass, tissue water potential, stomatal resistance and inorganic solute uptake were investigated in the mangrove, *Rhizophora mucronata* Lam. Plants were treated with solutions of seawater or PEG-6000 at osmotic potentials of $-0,05$, $-0,5$, $-1,2$ and $-2,4$ MPa for three weeks. Generally, effects of salinity and PEG-6000 were similar. Low external osmotic potentials decreased root mass, lowered tissue water potentials, increased stomatal resistance and influenced distribution of Na, K, Ca and Mg in tissues. The similarity of the responses of the plants to salinity and PEG-6000 suggests that the effects of salinity are mediated primarily through low osmotic potentials and not by salinity *per se*.

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Die uitwerking van verskeie konsentrasies van seewater en nie-soutbevattende poli-etileenglikol (PEG)-6000 van vergelykbare osmotiese potensiaal op droë-massa, waterpotensiaal van die weefsel, stomatêre weerstand, en op die opname van anorganiese opgeloste stowwe is in die wortelboom, *Rhizophora mucronata* ondersoek. Plante is 3 weke lank behandel met seewater- of PEG-6000-oplossings by osmotiese potensiale van $-0,05$, $-0,5$, $-1,2$ en $-2,4$ MPa. Oor die algemeen was die uitwerking van saliniteit en van PEG-6000 eenders. Lae uitwendige osmotiese potensiale het wortelmassa verminder, die waterpotensiale van weefsels verlaag, stomatêre weerstand verhoog en het 'n uitwerking gehad op die verspreiding van Na, K, Ca en Mg in die weefsels. Die ooreenstemming van die reaksies van die plante op saliniteit en PEG-6000 dui daarop dat die uitwerking van saliniteit primêr deur lae osmotiese potensiale bemiddel word, en nie deur saliniteit *per se* nie.

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G. Naidoo
Department of Botany, University of Durban-Westville, Private Bag X54001, Durban, 4000 Republic of South Africa

Introduction

Mangroves are halophytes which characterize the intertidal vegetation of many tropical and subtropical areas. Within intertidal zones soil salinities vary considerably, not only seasonally but also spatially. This variation is due to differences in the frequency of tidal inundation, evapotranspiration, water salinity and rainfall. Variations in salinity have important effects on plant water relationships (Flowers *et al.* 1977; Dainty 1977; Jefferies 1980; Ustin *et al.* 1982; Naidoo 1983; Naidoo 1985) and on osmoregulation (Stewart *et al.* 1979; Jefferies 1980; Cavalieri & Huang 1981; Abdul-Kadir & Paulsen 1982).

In intertidal zones plants are exposed to high salinities and low osmotic potentials. The effects of salinity on plant growth may be exerted through salt effects or osmotic effects. With few exceptions, little attempt has been made to separate salt effects from osmotic effects. Rozema (1979) reported little difference on the effects of mannitol and salt at the same osmotic potentials on proline accumulation in members of the Juncaceae and Gramineae. Cavalieri & Huang (1979), using polyethylene glycol (PEG)-4000 and NaCl on salt marsh plants, concluded that under certain conditions, NaCl exerted an osmotic effect as well as a specific salt effect. Responses of several halophytes to various concentrations of seawater and non-saline PEG-6000 were generally similar (Jefferies *et al.* 1979). Most of these studies were concerned primarily with the accumulation of organic solutes during osmoregulation in herbaceous, salt marsh perennials. The effects of salinity and low osmotic potentials on plant water relations and on the accumulation of inorganic solutes have received little attention. Mangroves occupy a delicate niche in our estuarine ecosystems and it is important that we understand the manner in which these woody perennials respond to marked changes in substrate salinity and osmotic potential.

The objectives of this study were to determine the effects of non-saline PEG-6000 and saline seawater solutions of comparable osmotic potential on dry mass, stomatal responses, tissue water potential and on the accumulation of inorganic solutes in the mangrove, *Rhizophora mucronata* Lam. PEG-6000 was chosen because it has been considered more suitable for work with plants than PEGs of lower molecular weight (Kaufmann & Eckard 1971; Michel 1971; Michel & Kaufmann 1973).

Methods

Plant culture

Propagules of *R. mucronata* were collected from the Sipingo estuary. The propagules were grown in 10-litre black plastic

buckets containing one-fifth strength complete nutrient solution (Hoagland & Arnon 1950). Nutrient solutions were renewed monthly. After 10 months plants were transferred to growth cabinets in 10-litre buckets. Photoperiod was adjusted to 12 h, light intensity to 200 $\mu E \ m^{-2}s^{-1}$, and temperature to 25°C (day) and 20°C (night).

After two months plants which had at least three pairs of mature leaves, were introduced gradually to solutions of low osmotic potential over a period of a week. At the end of the week all cultures were receiving appropriate treatments. Low osmotic potential solutions used were either different concentrations of seawater (0, 20, 50, 100%) or solutions of PEG-6000 which gave comparable osmotic potentials (−0,05, −0,5, −1,2, −2,4 MPa).

Prior to final adjustment of water potentials all cultures were fertilized with nutrient solution to give a final concentration of one-tenth strength complete solution. Osmotic potentials of solutions were monitored regularly and adjusted if necessary. Plants were cultured in the treatment solutions for three weeks after which they were harvested.

Water potential

Leaves were swabbed with distilled water to remove any excreted salts and blotted dry. Leaf discs, 5 mm in diameter, were removed from the middle of laminae of the third mature leaf. The discs were placed in Wescor C-52 sample chambers and left for 10 min for thermal and vapour equilibration. After a 10-s cooling period the water potential of three replicate samples for each treatment was measured with a Wescor HR 33T dewpoint microvoltmeter. The water potential of PEG or saline solutions was determined by applying the solution to filter paper discs which were then introduced into the sample chamber.

Stomatal resistance

Stomatal resistance was measured with a Lambda diffusive resistance autoporometer, model LI-65. Measurements were made at 11h00 on the third mature leaf of each plant. There were five replications per treatment. Resistance was determined for the abaxial surface because *R. mucronata* is hypostomatous.

Tissue element concentrations

Four plants from each treatment were harvested, the roots washed in tap water to remove adhering salts or PEG, then washed in distilled water. Plants were separated into roots and shoots, dried at 70°C, milled through a 2-mm screen and ashed (Ewel 1976). The ash was dissolved in 10N HCl filtered and made up to volume to yield a 0,1N HCl solution. Concentrations of Na, K, Ca and Mg were measured by atomic absorption spectrophotometry and expressed as a percentage of the dry weight.

Results

Growth

Plants in the −1,2 and −2,4 MPa treatments, both saline and PEG, exhibited symptoms of wilting two days after commencement of treatments. These symptoms were particularly severe in the −2,4 MPa treatments. After two weeks plants in the −2,4 MPa treatments developed symptoms of chlorosis and some of the leaves were in an advanced stage of abscission. The long hypocotyls of the plants were shrivelled and dehydrated. Water uptake appeared to be seriously affected by the −2,4 MPa treatment solutions.

Prior to introducing plants to treatments, four plants were harvested, dried, and root and shoot mass determined. These values are taken to represent the initial mass of the plants. After three weeks of treatment the dry mass was determined and this represents the final mass.

Plants in the control and −0,5 MPa saline treatments gained mass in roots and shoots (Table 1). In solutions of lower osmotic potential plants lost mass. Loss in mass was greater in roots than in shoots. In the −1,2 and −2,4 MPa saline treatments loss of root mass was 0,29% and 13,71% whereas corresponding values for the PEG treatments were 16,19% and 25,62% respectively, compared to the initial mass at the commencement of treatments. Therefore in the −1,2 and −2,4 MPa treatments loss of root mass was at least 10% greater in the PEG treatments.

Shoot mass was not as adversely affected as root mass. At lower salinities (−0,5 and −1,2 MPa) there was a gain in shoot mass. In the −2,4 MPa saline treatment there was a loss in shoot mass of 0,23%. All PEG treatments resulted in a loss in shoot mass, the losses ranging from 2,13% to 5,24%.

Water potential

In all treatments, tissue water potentials were at least 2 MPa below the osmotic potential of the external solution. The difference in water potential was lowest in the −2,4 MPa treatments. Tissue water potentials decreased as the external salinity or concentration of PEG increased (Figure 1). At all levels of external osmotic potential the lowering in tissue water

Table 1 Effects of various concentrations of seawater and non-saline PEG-6000 of comparable osmotic potential on dry mass (g) of *R. mucronata*. The means ± *SE* are given, $n = 4$

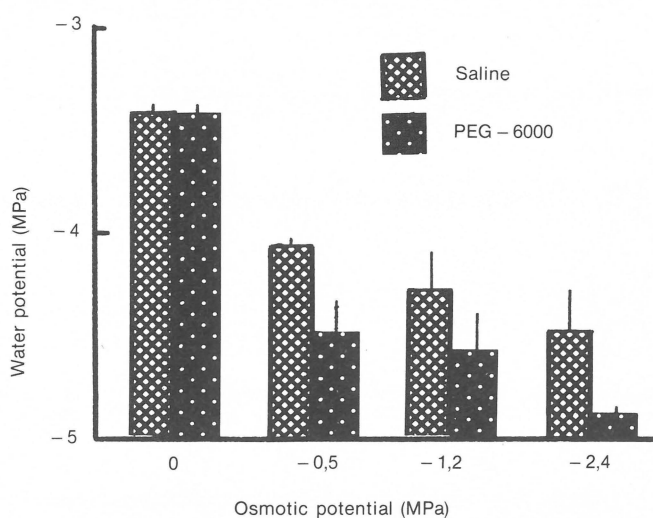| Solution osmotic potential (MPa)	Saline treatments		PEG treatments	
	Root	Shoot	Root	Shoot
initial	10,50 ± 0,7	61,80 ± 0,6	10,50 ± 0,7	61,80 ± 0,6
−0,05 (control)	11,4 ± 1,2	62,2 ± 1,7	11,4 ± 1,2	62,2 ± 1,7
−0,5	10,8 ± 0,3	64,9 ± 1,5	11,3 ± 0,8	60,3 ± 0,9
−1,2	10,5 ± 0,5	64,2 ± 1,1	8,8 ± 0,7	58,6 ± 1,7
−2,4	9,1 ± 0,6	61,7 ± 1,3	7,8 ± 0,4	60,5 ± 1,8

Figure 1 Effects of various concentrations of seawater and non-saline PEG-6000 of comparable osmotic potential on leaf water potential in *R. mucronata*. Vertical bars represent *SE*, $n = 3$.

potential was greater in the PEG solutions. The percentage lowering in tissue water potential in the $-0,5$, $-1,2$ and $-2,4$ MPa saline solutions was 18,3, 24,6 and 30,4 respectively; corresponding values for the PEG solutions were 29,0; 31,9 and 42,0%. An analysis of variance revealed no significant differences among treatments, probably because of insufficient replication.

Stomatal resistance

There was an increase in resistance with increase in salinity or concentration of PEG (Figure 2). Seawater concentrations at osmotic potentials of $-0,5$ and $-1,2$ MPa had little effect on resistance, the increase being 2,6 and 4,7% respectively. Saline solutions at $-2,4$ MPa resulted in a significant increase (62%) in resistance. PEG solutions of $-0,5$, $-1,2$ and $-2,4$ MPa significantly increased stomatal resistance by 39%, 72% and 97% respectively.
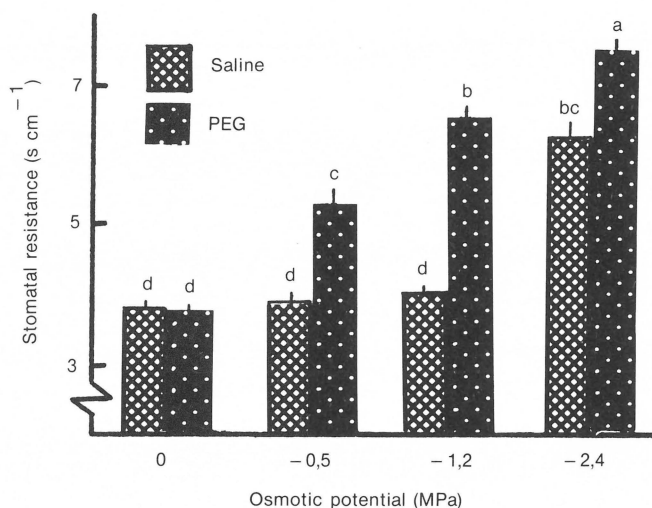

Figure 2 Effects of various concentrations of seawater and non-saline PEG-6000 of comparable osmotic potential on stomatal resistance in *R. mucronata*. Vertical bars represent *SE*, $n = 5$. Bars with the same letter are not significantly different at probability 0,05 using the Student – Neumann – Kuels Test.

Tissue element concentrations

Levels of Na in shoots and roots of the saline treatments increased with increasing salinity up to $-1,2$ MPa (Table 2). At $-2,4$ MPa salinity, there was greater accumulation of Na within roots, translocation to shoots being considerably reduced. In the PEG treatments levels of Na in shoots were generally similar. As the osmotic potential of the PEG solution decreased there was a tendency for Na to accumulate within roots. High levels of NaCl or PEG had no depressive effect on the uptake of K, Ca or Mg. In the saline treatments levels of K and Mg were higher than those in the PEG treatments. In both saline and PEG treatments there appeared to be greater retention of inorganic solutes within roots at the lower external osmotic potentials.

Discussion

Rhizophora displays a remarkable ability to tolerate extremely low external osmotic potentials (Naidoo 1985). The ability to osmoregulate efficiently is an adaptive feature of mangroves to the saline environment. Mangroves regulate the salt content by either excluding it from the roots or secreting salt from the leaves (Scholander *et al.* 1962). *Rhizophora mucronata* belongs to the former group and partially excludes salt from the xylem (Atkinson *et al.* 1967).

Water movement in the soil – plant – atmosphere continuum is dependent on the maintenance of a water potential gradient. Lowering of external osmotic potentials by saline or PEG solutions resulted in an adjustment in tissue water potentials. In all treatments tissue water potentials were at least 2 MPa lower than the osmotic potentials of the external solutions (Figure 1). Similar results were obtained for the mangrove *Avicennia marina* grown in saline solutions (Downton 1982). The maintenance of low tissue water potentials considerably below that of the external solution in *Rhizophora*, and by other mangroves in general, is probably an adaptive mechanism to prevent continual osmotic adjustment to rapid changes in external osmotic potential in intertidal areas. The mechanism by which internal osmotic adjustment occurs in *Rhizophora* is not known.

Lowering of tissue water potentials in *Rhizophora* was probably achieved by several mechanisms. The severe wilting symptoms observed with the $-2,4$ MPa solutions indicate that

Table 2 Effects of various concentrations of seawater and non-saline PEG-6000 of comparable osmotic potential on the concentration of elements in tissues of *R. mucronata*. Each value is the mean of 4 replications

Treatment	Tissue element concentration %							
	Na		K		Ca		Mg	
(MPa)	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Saline −0,05 (control)	0,33	0,15	0,34	0,62	0,02	0,12	0,03	0,02
−0,5	0,48	0,41	0,42	0,60	0,05	0,11	0,08	0,08
−1,2	0,50	0,75	0,55	0,93	0,03	0,10	0,05	0,09
−2,4	0,31	0,71	0,43	0,66	0,03	0,11	0,03	0,10
PEG −0,5 (control)	0,33	0,15	0,34	0,62	0,02	0,12	0,03	0,02
−0,5	0,33	0,25	0,68	0,41	0,06	0,13	0,07	0,05
−1,2	0,27	0,32	0,29	0,57	0,03	0,11	0,04	0,05
−2,4	0,40	0,30	0,49	0,68	0,03	0,13	0,04	0,04

tissues were partially dehydrated. This could have occurred as a result of osmotic reduction in water uptake. In the PEG treatments water flow through the plants was probably reduced by the plugging effect of PEG molecules in the free space of the roots (Erlandsson 1975). Partial dehydration of tissues in the −2,4 MPa solutions probably contributed significantly to the lowering of tissue water potentials.

Osmoregulation in some mangroves is achieved by the uptake of inorganic solutes (Popp 1983). In *R. mucronata* limited accumulation of Na, K, and to a lesser extent, Ca and Mg occurred (Table 2) but the concentrations of these elements in tissues were insufficient to account for the observed lowering of tissue water potentials. In addition, *R. mucronata* partially excludes salt at the level of the roots (Scholander *et al.* 1962).

Osmoregulation by the synthesis of low molecular weight organic solutes such as free amino acids and total methylated onium compounds occurs in salt marsh plants (Storey & Wyn Jones 1977; Jefferies *et al.* 1979; Cavalieri & Huang 1981) as well as in some mangroves (Wyn Jones & Storey 1981; Popp *et al.* 1984). Most of these organic solutes are nitrogen compounds. Reallocation of carbon and nitrogen resources to osmoregulation would severely limit plant growth rate. The marked loss in mass of plants, especially of root mass in both saline and PEG solutions could be attributed, at least partially, to the cost of organic osmoregulation. The severe reduction in root mass compared to shoot mass (Table 1) suggests that more of the plants' resources were directed to the shoot.

Lowering the osmotic potential of the external solutions resulted in increased stomatal resistance (Figure 2), especially in the PEG treatments. Solutions of low osmotic potential reduced water absorption and translocation and caused stomatal closure. PEG solutions had greater effects on stomatal resistance, as well as on water potential and final dry mass of plants than seawater concentrations of comparable osmotic potentials. This may be attributed to a greater reduction in water absorption as a result of the additive effects of low osmotic potential and high viscosity of the PEG solutions. In PEG solutions matric forces are the major component of water potential (Steuter *et al.* 1981). Reduction in water uptake, translocation and growth in PEG stressed plants were reported for wheat (Erlandsson 1975) and for bean, maize and sorghum (Kawasaki *et al.* 1983).

The possibility that PEG damaged membranes and entered plants cannot be excluded. Care was taken to ensure that roots were not damaged during culture of the plants. In the research literature effects of PEG on plant growth are contradictory. Some researchers reported that PEG has toxic effects on plants (Janes 1966; Leshem 1966). Lagerwerff *et al.* 1961 attributed toxicity of PEG-6000 to the presence of heavy metal impurities and suggested that these could be removed by dialysis or ion exchange. Lawlor (1970) considered that impurities were not responsible for toxic effects and suggested that PEG caused plant desiccation by blocking pathways of water movement. Others (Kaufmann & Eckard 1971) concluded that addition of PEG-6000 to nutrient solutions results in water relations similar to those expected in soil of the same water potential. In the present study, plants subjected to PEG treatments showed no visible signs of necrosis or death of leaves, suggesting that PEG did not enter leaves. These symptoms are characteristic of PEG within leaves (Lawlor 1970).

Although regarded as a salt excluder (Scholander *et al.* 1962), *Rhizophora* tends to accumulate Na and K as the salinity of the external solution increases (Table 2). Generally, in both saline and PEG treatments levels of K were higher than those of Na. At lower salinities Na was transported to the shoots which probably act as a sink. At higher salinities Na accumulated within roots probably as a result of reduced transpiration.

There appeared to be no depressive effect of salinity or PEG on uptake of K, Ca or Mg. There was a tendency for all elements to accumulate within roots at the lower osmotic potentials. Reduced transpiration at low external osmotic potentials, especially in the PEG treatments, probably decreased ion translocation.

The effects of various concentrations of seawater and non-saline PEG-6000 of comparable osmotic potential on dry mass, tissue water potential, stomatal responses and on the accumulation of inorganic solutes were similar. Low external osmotic potentials decreased root mass, lowered tissue water potentials and increased stomatal resistance. Trends in the distribution of Na, K, Ca and Mg seemed to be influenced by the osmotic potential of the external solution. Similar effects of NaCl and PEG on plants were reported previously for several salt marsh halophytes (Jefferies *et al.* 1979), for wheat (Erlandsson 1975), and for bean, maize and sorghum (Kawasaki *et al.* 1983). Although the patterns of response to saline and PEG treatments were similar, the magnitude was consistently different. This could probably be attributed to a greater reduction in water uptake as a result of the additive effects of low osmotic potential and high viscosity of the PEG solutions. The similarity of the responses of *R. mucronata* to NaCl and PEG, suggests that the effects of salinity are mediated primarily through low osmotic potential. The mechanism of internal osmotic adjustment in *R. mucronata* warrants further investigation.

Acknowledgements

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