

## Full Length Research Paper

# Effect of salinity on transmembrane potential of the roots of *Sesuvium portulacastrum* (L.) L.

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Halophytes are plants that complete their life cycle in saline environments. Halophytes can therefore be viewed as potential gene sources for genetic manipulation of economically important crop plants. In this study the influence of salinity on potential difference (PD) and surface pH of root cortical cells was investigated. The effect of the metabolic inhibitor, potassium cyanide (KCN), on PD of the root cells was also investigated. A study of the electrophysiological characteristics of the membrane of the root cortical cells showed that there were two components to the root cell PD. One component (diffusional) was affected by the increasing NaCl concentration in the growth medium, while the other component (electrogenic) remained unaffected by the NaCl concentration up to 200 mol m<sup>-3</sup>. There is evidence to suggest that this electrogenic component of the PD was maintained by an efflux pump which presumably controls ion uptake and indirectly growth. It is concluded that this electrogenic efflux pump is a major factor contributing to salt tolerance in *Sesuvium portulacastrum*.

**Key words:** Electrogenic pumps, halophyte, membrane potential difference, salinity, *Sesuvium portulacastrum*.

## INTRODUCTION

Salt tolerance is a gradual rather than a principal difference between plants (Flowers et al., 1977; Greenway and Munns, 1980). However, many questions remain concerning the mechanism which enables some species to grow at high salinities (e.g. halophytes), while others (glycophytes) die at even low salinities.

The main characteristic of salt tolerance may be ascribed to the ability to acquire sufficiently cheap osmotic ions in order to avoid drought stress, and still maintain a strong coupling between growth rate and ion uptake, to avoid cytoplasmic toxification from excessive ion influx (Flowers et al., 1977; Lv et al., 2008). Biomass production at saline conditions depends on the ability to

maintain high net photosynthesis at minimal water loss and energy consumption (Alvarez et al., 2012; Naidoo et al., 2012).

It is thought that transport of ions across the root involves three major steps, viz., transport across the plasmalemma of epidermal and cortical cells, transport through the symplast, and subsequent release into the xylem vessels (Pitman, 1977). In the first step it is widely accepted that electrogenic proton pumps play a central role (Jeschke, 1980; Poole, 1978). One of the driving forces for the transport of ions from cortex to the shoot symplasm is the trans-root potential (TRP, that is, the potential difference between the external medium and the

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xylem sap (Dunlop and Bowling, 1971). Like the membrane potential, the TRP has a passive (diffusional) and an active (electrogenic) component. The best criterion for the presence of an electrogenic pump is the generation of a membrane potential which is greater than that predicted by the Goldman equation (Bowling, 1976).

The effect of metabolic inhibitors has been suggested as another criterion for the presence of electrogenic pumps. This was applied to epicotyl and coleoptile cells by Higinbotham et al. (1970). The argument put forward here is that if an inhibitor such as dinitrophenol (DNP) or cyanide causes a rapid depolarization of the potential, then it is affecting an electrogenic component of the transport process. In the absence of an electrogenic pump the inhibitor would be expected to cause a gradual fall in the potential as diffusion potential was slowly dissipated due to evening out of the ion concentrations on each side of the membrane after the cessation of active transport (Bowling, 1976).

The  $H^+$ -ATPase is the major electrogenic pump in the plasma membrane (PM) of the plant cells (Zhang et al., 2014). It should be noted that by pumping protons from the cytoplasm to the apoplast it generates an electrochemical proton gradient, which drives the transport of mineral ions and organic solutes, and plays a crucial role in cytoplasmic and apoplastic pH homeostasis. The plasma membrane  $H^+$ -ATPase (PM- $H^+$ -ATPase) participates in a variety of physiological processes such as phloem loading, stomata opening, mineral nutrition, growth of root hairs and pollen tubes, salt and osmotolerance, leaf movements, and acid growth (Arango et al., 2003; Sondegaard et al., 2004; Zhang et al., 2014).

*Sesuvium portulacastrum* (L.) L (seapurslane) is one of the fast growing herbaceous, perennial, dichotomous halophyte belonging to the family Aizoaceae (Lokhande et al., 2009). It grows as sprawling perennial herb up to 30 cm high, with thick, smooth stems up to 1 m long. It has smooth, fleshy, glossy green leaves that are linear or lanceolate, from 10 to 70 mm long and 2 to 15 mm wide. Flowers are pink or purple (Prescott and Venning, 1984).

In this study the influence of salinity on potential difference (PD) and surface pH of root cortical cells was investigated. The experiments were performed on both excised and intact roots (to determine whether transport of assimilates from the shoot has an effect on PD using a special microscope stage assembly (Graham and Bowling, 1977). The effect of the metabolic inhibitor, potassium cyanide (KCN), on PD of the root cells was also investigated.

## MATERIALS AND METHODS

### Cultivation

Stem cuttings of *S. portulacastrum* (stock plants) were cultivated under greenhouse conditions in plant pots containing vermiculite. They were watered daily with tap water and once weekly

supplemented with 'phostrogen' (plant food). Plant cuttings were taken from stock plants and allowed to root after applying a rooting hormone to the cut end. The cuttings were kept in a growth room under continuous light conditions and a mean temperature of 24°C. These were watered once in three days with one tenth strength of Jensen and Pettersson (1984) culture solution.

After a period of two weeks, the cuttings were transferred into culture solution containing various concentrations of NaCl. Seven plant pots were used and each pot contained four plants. Plants were subjected to treatments of 0, 100, 200, 400 and 600 mol m<sup>-3</sup> NaCl for two and half months. The plants were grown at 24°C under continuous light from warm-white fluorescent tubes giving a mean light intensity of 206 μmol m<sup>-2</sup> s<sup>-1</sup> and at about 65% relative humidity. Higher salinities were gradually raised until the required concentration was attained to avoid any osmotic shock on the plants. The concentration was increased by 100 mol m<sup>-3</sup> NaCl after every three days. Solutions were changed on a weekly basis.

### Determination of dry weight

Plants from various NaCl concentrations were separated into roots and shoots. The parts were washed with distilled water and blotted dry with a paper towel. The plant parts were then dried in an oven at 80°C for 48 h for dry weight determination.

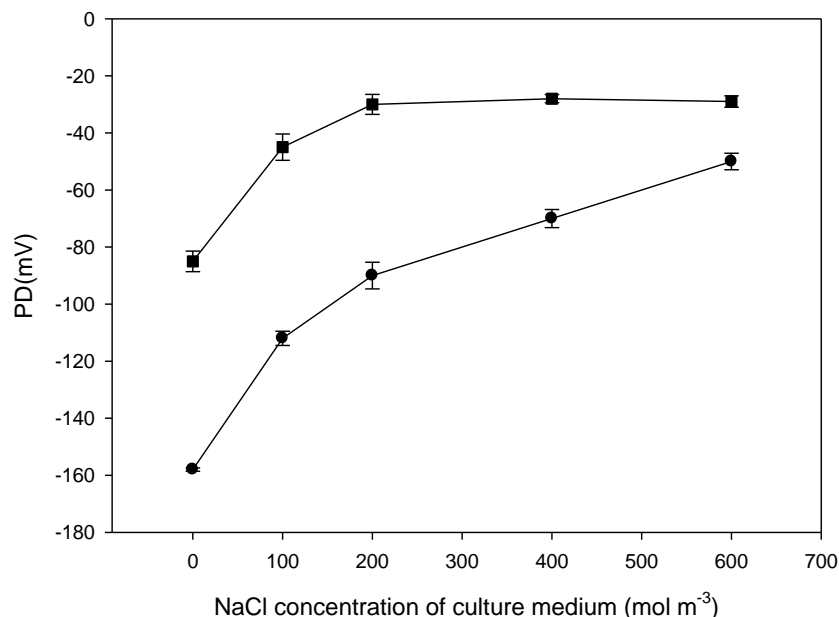
### Transmembrane potential (PD) of root cortical cells

Plants were grown hydroponically in various NaCl concentration (0, 100, 200, 400 and 600 mol m<sup>-3</sup>) as previously described (under cultivation section). PD measurements were determined according to the method employed by Graham and Bowling (1977), as described below. A flow-through cell was designed to fit on the stage of a microscope on either side of which were micro-manipulators for interesting microelectrodes into cells. The flow-through chamber was fed on the side by a peristaltic pump from a reservoir of culture solution and on the other outflow channel extended over the vessel which contained the plant root system.

A single root was led from the culture vessel along the outflow channel and fixed in place under the microscope between two cover-slips (note, for excised roots, PD was determined by cutting about 20 mm of root segment from the apex, fixed as described above). The two cover-slips formed the top of the chamber which has no end walls, its volume of culture solution held in place by surface tension. Thus a flow of solution of 86 ml h<sup>-1</sup> was maintained while still allowing access to horizontally moving microelectrodes. The whole of the experimental root was kept moist in the chamber and outflow channel, the solution finally running down the root into the culture vessel. The experimental plants were exposed to photon flux density of 262 micromoles m<sup>-2</sup>s<sup>-1</sup> and measurement of the PD began at least 30 min after setting the plant in the apparatus or after excision of segments.

The bathing solution used during the electrophysiological measurements were equivalents of the culture solutions (that is, culture solution containing the required NaCl concentrations) in which the plants were grown. The sensing and reference electrodes were Ag/AgCl half cells. Both electrodes were filled with a 3000 mol m<sup>-3</sup> KCl in 1% agar salt bridge. A length of borosilicate glass tubing (Clark Electromedical Instruments) of 2 mm outside diameter was cut into 5 cm lengths. The 5 cm length glass tubing was pulled on a Palmer vertical microelectrode puller to give a tip diameter of less than 1 μm. It was then filled with 3000 mol m<sup>-3</sup> KCl in 1% agar salt bridge.

As Graham and Bowling (1977) observed, tip potentials tended to become more negative with use, due to contamination of the tip with cellular constituents and as such microelectrodes were discarded when tip potentials became more negative or unstable.



**Figure 1.** Effect of NaCl on transmembrane potential of intact (●) and excised (■) roots of *S. portulacastrum*. Vertical bars represent SEM (n=3).

The test microelectrode was inserted into the cortical cells (in the vacuole) using Zeiss micromanipulators (Carl-Zeiss, Jena, G.D.R) and allowed to rest in one of the three outer layers of cells. The PD between the vacuole and external solution was measured using a PITMAN 437 electrometer connected to a BRYANS chart recorder (model 27000). The measurements were made on mature cells, about 10 mm from the root apex. For respiration blocking experiments, potassium cyanide (KCN) was added to the appropriate bathing solution to give a concentration of 1 mol m<sup>-3</sup> KCN.

#### Profile of root surface pH

pH microelectrodes were fabricated as described by Bowling (1989). The method basically involved taking a length of borosilicate glass tubing of 2 mm outside diameter which was cut into 5 cm lengths. Each length was used to pull two micropipettes on a C.F Palmer vertical electrode puller (Searle Bioscience, Sheerness, Kent U.K). The micropipettes were then placed, tips upward, in a suitably sized hole drilled in an aluminium block. The block was then put on a Petri dish and covered with an inverted beaker (250 ml).

The dish and its contents were placed in an oven at 200°C for an hour in order to dehydrate the glass of the micropipettes. About 2 ml of dimethyldichlorosilane (2% in 1,1,1-trichloroethane), BDH chemicals Ltd, Poole, England) were drawn into a Pasteur pipette and released under the inverted beaker. The silane vaporized and coated the tips of the microelectrodes. These were left in the oven for a further one hour at 200°C to allow the silane to bake onto the surface of the glass in order to waterproof the glass (Edwards and Bowling, 1986; Edwards et al., 1988).

On cooling, the microelectrodes tips were back-filled with a pH-sensitive liquid-ion-exchanger (LIX) and the stems filled with 3000 mol m<sup>-3</sup> KCl (Edwards and Bowling, 1986; Edwards et al., 1988). The pH-LIX was made up according to Bowling (1989): 1.22 ml tri-n-dodecylamine, 5.93 ml 2-nitrophenol octyl ether and 0.07 g sodium tetraphenyl borate. As this is an organic solvent based

compound the silanisation of the glass prevents the LIX being displaced from the microelectrode tip when the microelectrode was placed in aqueous solutions (Bowling, 1989). The pH and reference microelectrodes were connected via Ag/AgCl half-cells to the FD223 electrometer, pH on channel A and reference on channel B, the electrometer displaying A-B that is, the voltage corresponding to the pH at the pH microelectrode tip.

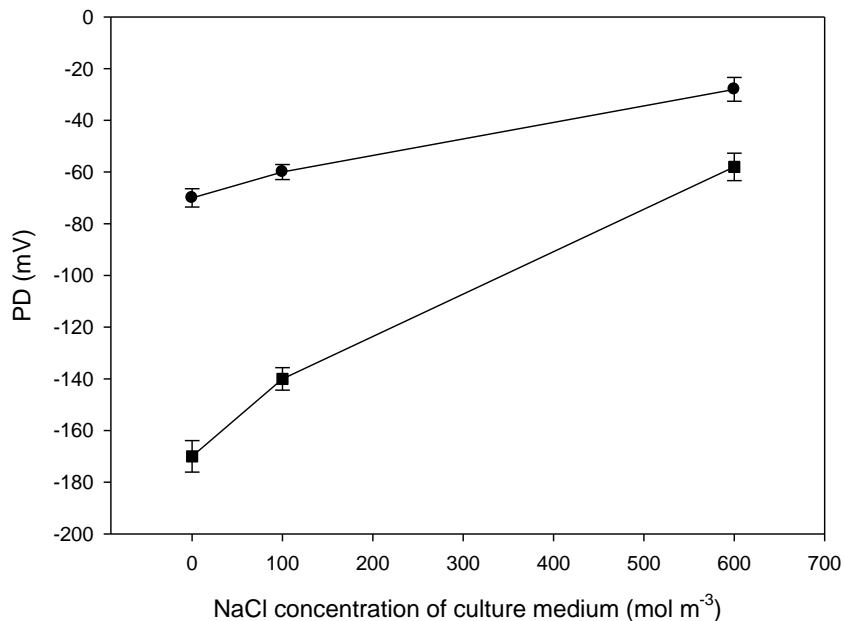
For this calibration curve, pH buffer solutions of pH ranging between 5 and 9 were used. Both pH and reference microelectrodes were placed in the pH buffer solution. The slope of the calibration curve between pH 5.0 and 9.0 was about 60 mV per pH unit. Once the slope has been determined, the electrometer was adjusted to display the signal directly in units of pH as with a conventional pH meter. Reference microelectrodes were made by filling non-silanised micropipettes with 3000 mol m<sup>-3</sup> KCl.

The root surface pH profile was determined by placing the pH microelectrode tip close to the root surface, while the reference microelectrode remained in the bathing solution. The root surface pH was measured while the root was attached to the plant. Measurements were taken from the root apex back to at least 13 mm along the length of the root.

## RESULTS

### Transmembrane PD of root cortical cells

Figure 1 shows measurements of the transmembrane PD of cortical cells of both attached and excised roots of *S. portulacastrum*. In all instances, it can be seen that the PD tended to decrease with increasing NaCl concentration of the growth media. The excised root segments appeared to have a lower transmembrane potential than that of the intact roots. The PD of the excised roots was rapidly depolarized as NaCl concentration of external media was raised from 0 up to



**Figure 2.** The effect of KCN on intact root cell PD (■ without KCN; ● with KCN). Vertical bars represent SEM (n=3).

200 mol m<sup>-3</sup>. At higher salinities, the PD of excised roots remained more or less constant.

### Effect of potassium cyanide (KCN) on transmembrane potential of root cortical cells

The effect of KCN on the transmembrane potential of the roots of *S. portulacastrum* is shown in Fig. 2. It is worth noting that, potassium cyanide (KCN) appeared to depolarize the membrane of the intact roots to more or less equal magnitude to those observed for the excised roots (Figure 1). This indicated the presence of an electrogenic component sensitive to both KCN and excision. In all cases, KCN reduced the transmembrane potential to a basal PD. This basal PD could be equated with a diffusion component to the overall PD. It can be seen that this residual diffusional PD was affected by NaCl concentration in the culture solution, reducing it from -75 to -26 mV at 0 and 600 mol m<sup>-3</sup> NaCl, respectively (Figure 2).

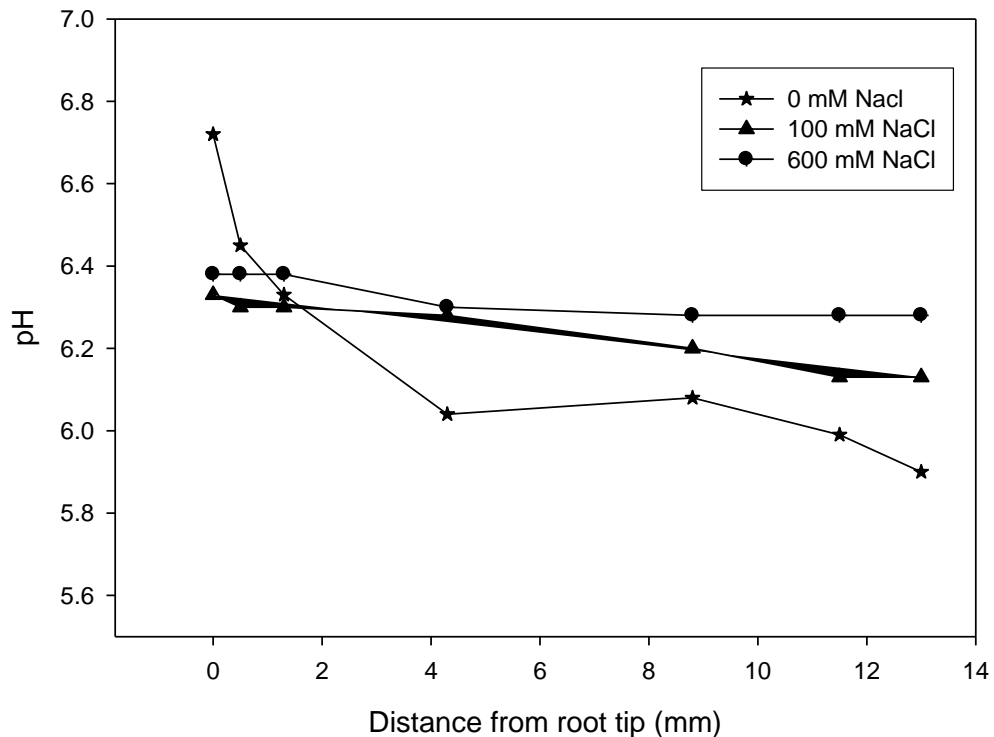
### Root surface pH profile

Figure 3 shows the surface pH profile of roots growing at various NaCl concentrations of the culture solution. At 0 mol m<sup>-3</sup> NaCl concentration of the culture solution, the pH of the solution was 6.31. The surface pH at root apex was 5.89 and it decreased further back along the root length. It reached a pH value of 5.48 at a distance of 13 mm from the root apex.

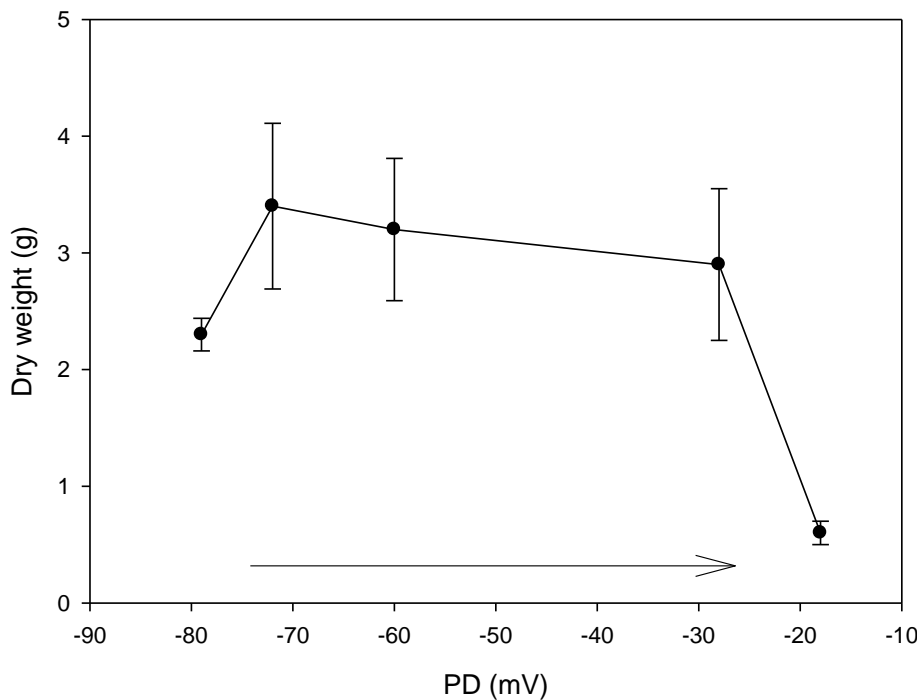
A similar trend was observed when root surface pH of plants grown in 100 and 600 mol m<sup>-3</sup> NaCl was determined. However, in these two instances, pH difference between that of culture solution and root surface was relatively small. It can be seen that 100 mol m<sup>-3</sup> NaCl concentration exhibited a pH of 6.33 and this was reduced to pH 6.30 at the root apex. It was further reduced to pH 6.12 at 13.0 mm away from the root tip. It appeared that root surface pH of plants grown in 600 mol m<sup>-3</sup> NaCl concentration of the culture solution was more or less the same as that of the bathing medium (Figure 3). When the concentration of NaCl in the culture solution was 600 mol m<sup>-3</sup>, the pH was 6.38 and there was only a pH drop of 0.1 at 13.0 mm back along the root length from the root apex. It appeared that as the NaCl concentration of the culture solution increased, the difference between the root surface pH and that of the bathing medium decreased until it became almost negligible.

### Electrogenic PD of root cortical cells and plant growth

The relationship between the electrogenic (cyanide sensitive) component of the transmembrane potential of root cortical cells and the dry weight of *S. portulacastrum* is shown in Figure 4. The arrow in Figure 4 indicates the direction of increasing NaCl concentration of the culture solution. The electrogenic component of the PD decreased with increasing NaCl concentration of the external medium. It appeared that growth decreased with



**Figure 3.** The effect of NaCl concentrations (0, 100 and 600 mol m<sup>-3</sup>) of the external medium on root surface pH. The pH at 0 mm corresponds to the pH of the culture solution containing the above respective NaCl concentrations.



**Figure 4.** The relationship between growth and electrogenic component of the root transmembrane potential. The arrow indicates the direction of increasing NaCl concentration of the growth media. Vertical bars represent SEM (n=4).

decreasing electrogenic component of the transmembrane of the root cortical cells. However, at -79 mV, growth was relatively poor, despite the high value of the electrogenic component. It should be borne in mind that this point corresponds to plants grown in 0 mol m<sup>-3</sup> NaCl concentration of the culture solution.

## DISCUSSION

### Transmembrane potential of root cortical cells

The results obtained in this study are in agreement with those obtained by Graham and Bowling (1977) and Anderson et al. (1977), which indicated the existence of two components to the root cell PD. It appeared that there was a component of the PD in attached roots which was lost on excision, leaving a basal potential difference. The component of the PD which disappeared after excision appeared to depend on metabolic activity, as cyanide (a metabolic inhibitor) completely removed it (Figure 3). This component would appear to be due to the activity of an electrogenic pump as defined by Higinbotham et al. (1970). This PD may be referred to as the electrogenic component of the PD due to the activity of an electrogenic pump (Zhang et al., 2014; Arango et al., 2003; Sondegaard et al., 2004).

There is further evidence to suggest that this component of the PD was metabolically derived. For instance, Graham and Bowling (1977), observed that in sunflower roots, in darkness, the PD fell to approximately the basal level. This can be ascribed to a reduction in photosynthates supply from the leaves. This would in turn reduce the activity of the electrogenic pump. This appears to be analogous to the excision effect observed in segments by Pitman et al. (1970) and Graham and Bowling (1977). The excision effect on PD was also observed in this study (Figure 3). These results have demonstrated that the root cell PD should be determined with the roots attached to, rather than excised from the plant.

The highest NaCl concentration (600 mol m<sup>-3</sup>) of the culture solution reduced the PD to about 130 mV indicating that the salt may have been affecting both components of the PD. However, the electrogenic component of the PD remained unaffected by NaCl concentration in the external media up to 200 mol m<sup>-3</sup> (Figure 4), while the passive diffusional component was greatly affected by NaCl. This is in accord with Graham and Bowling's (1977) suggestion that the diffusional component is highly dependent on the external concentration.

The relative insensitivity of the electrogenic pump to NaCl concentrations up to 200 mol m<sup>-3</sup> in the roots of *S. portulacastrum* might account for the salt tolerance in this species. These results are in agreement with those obtained by Niu et al. (1993) which demonstrated that the

plasma membrane H<sup>+</sup>-ATPase (PM-H<sup>+</sup>-ATPase) gene expression in the halophyte *Atriplex nummularia* was more responsive to NaCl than in the glycophyte tobacco. Similarly, Yang et al. (2004) found that the salt stress increased the PM-H<sup>+</sup>-ATPase activity in roots of salt-tolerant wheat L-Ch20, but reduced the PM-H<sup>+</sup>-ATPase activity in salt-sensitive wheat Y-J24.

### Root surface pH

It has been proposed that the electrogenic component of the PD is related to the active H<sup>+</sup> efflux at least in fresh water giant algal cells (Spanswick, 1974). H<sup>+</sup> efflux may be accompanied by influx of another cation and Poole (1974), observed a correlation between the PD and influx of K<sup>+</sup> in beetroot tissue. The difference in pH between that of the bathing solution and that at the root surface might suggest the presence of an electrogenic efflux pump (Figure 3).

There appeared to be a higher concentration of H<sup>+</sup> ions close to the root surface than in the bathing solution. If this is due to the activity of the proton efflux pump, it would appear that increasing NaCl concentration of the growth medium has an effect on it, as evidenced by the relatively small drop in pH between pH at root surface and that of the bathing solution (Figure 3).

As the activity of the pump is reduced at high salinities, this would inevitably reduce uptake of cations such K<sup>+</sup> as the electrochemical gradient will be reduced. This should also reduce growth (Figure 4) not only by reducing salt uptake, but also through an adverse effect on osmotic gradient between the cells and the external solution (Carkilar and Bowling, 1981). These results have suggested a role of the H<sup>+</sup> efflux pump in maintaining the PD, in agreement with previous findings (Zhang et al., 2014).

### Electrogenic PD of root cortical cells and growth

It appears that an electrogenic pump might make a major contribution to growth and that the activity of the pump is inhibited by high concentrations of NaCl (above 200 mol m<sup>-3</sup>), thus resulting in reduced growth of the plant (Figure 4), consistent with results obtained by Rajaravindran and Natarajan (2012), who observed a marked decrease in the growth of *S. portulacastrum* at NaCl concentration above 600 mM.

There is evidence to suggest that this electrogenic component of the PD is maintained by a H<sup>+</sup> efflux pump (Zhang et al., 2014), which presumably controls ion uptake (Arango et al., 2003; Soodegaard et al., 2004) and indirectly growth (Figure 4). These results are in agreement with those obtained by Abideen et al. (2014) who observed increase in growth of *P. karka* at moderate salinity (ca. 100 mM NaCl).

Yang et al. (2004) found that the salt stress increased the PM-H<sup>+</sup>-ATPase activity in roots of salt-tolerant wheat L-Ch20, but reduced the PM-H<sup>+</sup>-ATPase activity in salt-sensitive wheat Y-J24. It is therefore, suggested that an electrogenic efflux pump is a major factor contributing to salt tolerance in *S. portulacastrum*, as a halophyte.

## Conflict of Interests

The author have not declared any conflict of interests.

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