

Short Communication

Evidence for the presence of two components of the root transmembrane potential of a halophyte *Sesuvium portulacastrum* (L) L grown under saline conditions

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This study has shown that the root cell potential difference (PD) has two components. One component (diffusional) was unaffected by the increasing NaCl concentration in the growth medium, while the other component (electrogenic) was affected by the NaCl concentration mostly above 200 mol m⁻³. The component of the PD which disappeared after excision appeared to depend on metabolic activity, as cyanide (a metabolic inhibitor) completely removed it. This component would appear to be due to the activity of an electrogenic pump, possibly, powered by ATP.

Key words: *Sesuvium portulacastrum*, salinity, electrogenic pumps, halophyte.

INTRODUCTION

Salt tolerance is 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 (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).

Transport of ions across the root involves three steps, viz., and transport across the plasmalemma of epidermal and cortical cells, transport through the symplast and subsequent release into xylem vessels (Pitman, 1977). It is widely accepted that electrogenic proton pumps play a central role in transporting ions across the plasmalemma (Poole, 1978; Vera-Estrella et al., 2005).

Diffusion potentials depend on the difference in permeability of the membrane to different ions (Bowling, 1976). The diffusion potential across a membrane can be described by an expression derived by Goldman (1943) usually referred to as the Goldman equation. In some tissues there is evidence for a component of the membrane potential which is not explicable in terms of diffusion. Active transport systems may make a contribution to the membrane potential by the transfer of

charges across the membrane. Such transport is termed 'electrogenic' and is described as being brought about by 'electrogenic pumps'. 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. Curious aging effects on the potential difference have been reported which appear to be due to excision of the tissue (Pitman et al., 1970).

In this study the influence of salinity on potential difference (PD) on both excised and intact roots of *Sesuvium portulacastrum* was investigated. The effect of the metabolic inhibitor, potassium cyanide on PD of root cells was also assessed.

MATERIALS AND METHODS

Stem cutting 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). For experimental purposes, plant cuttings were taken from the stock

plants, allowed to root for two weeks in vermiculite in plant pots. 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. Plants were grown hydroponically in various NaCl concentrations (0, 100, 200, 400 and 600 mol m⁻³). The plants were grown at 24°C under continuous light from warm-white fluorescent tubes giving a mean light intensity of 206 μmol m⁻² s⁻¹ and at about 65% relative humidity. Higher salinities were gradually raised until the required concentration was attained to avoid any osmotic shock on plants. The concentration was increased by 100 mol m⁻³, after every three days. Solutions were changed on weekly basis.

Figure 1. Effect of NaCl on the transmembrane potential of intact and excised roots of *S. portulacastrum*. Vertical bars represent SEM (n = 10).

Transmembrane potential of root cortical cells

PD measurements were determined according to the method employed by Graham and Bowling (1977). A flow-through cell was designed to fit on the stage of a microscope on either side of which were micromanipulators for inserting microelectrodes into cells. The flow-through chamber was fed on one side by a peristaltic pump from a reservoir of culture solution and on the other an 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 had no end walls, its volume of culture solution held in place by surface tension. Thus a flow of solution of 86 ml h⁻¹ 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 262-

μmol m⁻² s⁻¹ and measurements of PD began at least 30 min after setting the plant in the apparatus or after excision of segments.

The bathing solutions used during the electrophysiological measurements were equivalents of the culture solutions (i.e., 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⁻³ 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⁻³ KCl. 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 microelectrode were discarded when tip potentials became more negative or unstable.

The test microelectrode was inserted was inserted into the cortical cells (in the vacuole) using Zeiss macromanipulators (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, KCN was added to the appropriate bathing solution to give a concentration of 1 mol m⁻³ KCN.

RESULTS AND DISCUSSION

Figure 1 shows transmembrane potential (PD) of root cortical cells of both attached and excised roots of *S. portulacastrum*. The PD tended to decrease with increasing NaCl concentration of the growth media. The excised roots 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 the external media was raised from 0 up to 200 mol m⁻³. At higher salinities, the PD of excised roots remained more or less constant.

The time-trend in transmembrane potentials in an intact root of a plant growing in 100 mol m⁻³ NaCl in culture solution, following the application of and the withdrawal of 1 mol m⁻³ potassium cyanide (KCN) supplied via the external culture solution is shown in Figure 2. KCN depolarized the membrane from -138 mV to a basal level of -58 mV. After the removal of KCN, the membrane potential was restored, even though the PD did not fall to the initial value of -138 mV.

The results obtained in this study indicated that the membrane potentials of plant cells have two components: a diffusional potential and a component resulting from electrogenic ion transport (Bowling, 1977; Anderson et al., 1977; Spanswick, 1981).

It appeared that there was a component of the PD in attached roots which was lost on excision, leaving a basal potential. The component of the PD which disappeared after excision appeared to depend on metabolic activity, as cyanide (a metabolic inhibitor) completely removed it (Figure 2). 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

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Figure 2. The time-trend in transmembrane potential of root cortical cells of plants grown in 100 mol m⁻³ NaCl before the application (1) of and subsequent withdrawal (2) of 1 mol m⁻³ KCN.

referred to as the electrogenic component of the PD due to the activity of an electrogenic pump, possibly, powered by ATP (Taiz and Zeiger, 2006).

There is evidence to suggest that this component (electrogenic) 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 (assimilates) supply from leaves. This would in turn reduce the activity of the electrogenic pump. This appears to be analogous to the excision effect observed in this study (Figure 1) by Pitman et al. (1970) and Graham and Bowling (1977). The results in this study have demonstrated that the root cell PD should be determined with the roots attached to, rather than excised from the plant.

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