

Proton Efflux from Roots of Intact *Spergularia marina* Plants

JOHN M. CHEESEMAN¹ AND CAROL ENKOJI

Department of Plant Biology, University of Illinois, 505 S. Goodwin Avenue, Urbana, Illinois 61801, U.S.A.

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ABSTRACT

Despite numerous considerations of the importance of proton extrusion to plant mineral nutrition, measurements of net H⁺ efflux rates in photo-autotrophic plants growing in complete medium are rare, and the rates of efflux reported are considerably lower than that typical of low-salt seedlings. We present here the first report of short term net proton efflux from roots of a halophyte, and the highest rate of net efflux reported to date for any higher plant, in both low-salt and complete nutrient media. We also show responses of that efflux to a variety of treatments, including NaCl fusicoccin, shoot excision and darkness.

Key words: Halophyte; Sea spurrey; H⁺ efflux; Roots.

INTRODUCTION

Though the relationship between H⁺ fluxes and the fluxes of other ions has been a question of interest to physiologists for more than 100 years (Rautenberg and Kuhn, 1864), for the past 25 years, quantitative studies on H⁺ movements in higher plant roots have been almost entirely restricted to low-salt, excised roots. The paucity of data concerning the relationship between H⁺ efflux and, for example, K⁺ influx in higher plants growing on complete nutrient medium might be explained in part by the fact that H⁺ efflux, K⁺ stimulation and response to such agents as fusicoccin (FC) are difficult to demonstrate under such conditions. Glass, Siddiqi, and Giles (1981) surveyed 24 varieties of barley growing on dilute complete medium, finding maximum rates of H⁺ efflux of less than one-third the rates in low-salt plants. Under complete nutrient conditions, many varieties showed virtually no net H⁺ efflux. Bowling, Graham, and Dunlop (1978) reported net H⁺ efflux as part of a larger study using intact sunflower plants. Maximal rates of approximately 1.7 $\mu\text{mol g}^{-1}$ fr. wt. root h⁻¹ were highly sensitive to loss of photosynthate supply. Hanson and Clarkson (personal communication) found very low rates of efflux from root segments of etiolated barley seedlings grown in complete nutrient medium, and no response to K⁺ or FC. Short-term H⁺ efflux from 2-week old, light grown, complete nutrient corn (*Zea mays*) seedlings was immeasurable using the standard medium for low-salt root studies (Cheeseman, unpublished observation).

In this paper, we present data showing rapid net H⁺ efflux from roots of the salt marsh halophyte, *Spergularia marina* Griesb. (Caryophyllaceae) grown on complete nutrient medium in the light. Proton efflux occurs into either the standard low-salt medium or into the complete nutrient medium, and it is sensitive to darkness, shoot excision, and FC. So far as we know, this is the first report of H⁺ fluxes in a halophyte, and the rates reported are the highest to date for any higher plant.

¹ To whom correspondence should be addressed.

Abbreviations: FC- fusicoccin; DCCD- dicyclohexylcarbodiimide.

MATERIALS AND METHODS

Seeds of *Spergularia marina* were collected initially from the salt marsh at Kincardine Bridge on the Forth River in Scotland in October, 1979. For experiments, seeds from a single plant grown for seed production were swept from the growth chamber bench onto vermiculite and watered once with a complete nutrient medium equivalent to $0.6\times$ sea-water (sodium free) supplemented with nitrogen, phosphorous and micro-nutrients (Johanson and Cheeseman, 1983), and daily thereafter with distilled water. Two-week-old seedlings were transferred to 1.0 cm thick styrofoam islands floating on aerated nutrient medium. Growth chamber conditions were as previously described (Johanson and Cheeseman, 1983). Unless otherwise stated, a fresh water growth medium was used (equivalent to $0.1\times$ sodium-free modified sea water). Plants were used for experiments at approximately 4 weeks after transfer to solution culture.

Proton efflux was measured with a pH electrode and calculated on the basis of quantity of hydroxyl required to return the solution to the original pH after a period of measurement. In order to prevent possible adverse effects of maintaining plants in small volumes of solution for periods of up to 8 h, a plexiglas chamber was constructed with a solution volume of approximately 100 cm^3 . The pH electrode and aeration inlet were placed in the chamber and the chamber was connected to a reservoir of 2.0 dm^3 volume through a peristaltic pump. The pump was operated for 20 min to circulate solution, then stopped for 15–50 min as required to measure efflux. Before restarting the pump, back titration with NaOH was performed; pH was maintained in this way between 6.9 and 6.6. All changes in pH were recorded on a chart recorder. The reservoir and chamber were maintained in a water bath at 26°C and illuminated with a tungsten flood lamp. Light was filtered through 4.0 cm water and 1.0 cm of plexiglas; intensity at the leaf level was $500\text{--}550\ \mu\text{mol quanta m}^{-2}\text{ s}^{-1}$.

Two media were used for pH recordings. The 'low-salt' medium contained (in mol m^{-3}) CaSO_4 , 0.2; K_2SO_4 , 0.1; K_2HPO_4 , 0.03; adjusted to an initial pH of 6.8. The 'complete' medium was the same composition as the growth medium with the exception that HCO_3^- was replaced by Cl^- and $\text{NH}_4\text{H}_2\text{PO}_4$ was reduced to 0.24 mol m^{-3} . These changes were made to reduce the buffering capacity and make H^+ efflux measurable.

In the case of fusicoccin treatment, circulation was stopped entirely following its addition. The final concentration of fusicoccin was 10 mmol m^{-3} (0.5% ethanol). In detopping experiments, the shoot was removed with a sharp, double edged razor blade at the top of the hypocotyl. For darkness, the light was turned off and the entire water bath apparatus enshrouded in a black cloth; bath temperature was not altered.

Following recordings, plants were harvested, blotted and weighed. Results are expressed on the basis of root fresh weight.

RESULTS

The mean net proton efflux rate from fresh water grown plants into standard low-salt medium was $9.2 \pm 2.6\ \mu\text{mol g}^{-1}\text{ fr. wt. h}^{-1}$ (90 determinations; 21 experiments); into the balanced nutrient medium it was 9.6 ± 1.7 ($n = 25$; 6 experiments). The means were not statistically different. The greatest net flux measured in a single experiment (in the absence of FC) was $23.6\ \mu\text{mol g}^{-1}\text{ fr. wt. h}^{-1}$. This rate was maintained for more than 1 h. A similar maximal rate was found following FC addition to an 'average' experiment. Net flux was also measured using plants grown on $0.2\times$ and $0.4\times$ sea water, both into the standard medium and into a medium of equal salinity to the growth medium. Those rates were 30–60% of the fresh-water average.

Figure 1 shows some of the characteristics of the H^+ efflux in *S. marina*. In the absence of manipulation, a steady efflux was maintained for more than 200 min (Fig. 1a). Proton efflux responded to darkness with a gradual decline (Fig. 1b), often to zero over a period of approximately 2 h. Shoot excision also resulted in a rapid decline of efflux which was sometimes partially restored with addition of 25 mol m^{-3} dextrose (Fig. 1c, d). FC stimulated H^+ efflux immediately after addition, even in excised roots (Fig. 1c–e). DCCD rapidly eliminated efflux (example not shown). Addition of NaCl to the equivalent of $0.1\times$ seawater (45 mol m^{-3}) had no effect (Fig. 1d). Plants grown on saline medium ($0.2\times$ seawater, Fig. 1e) responded in a fashion similar to fresh-water grown plants.

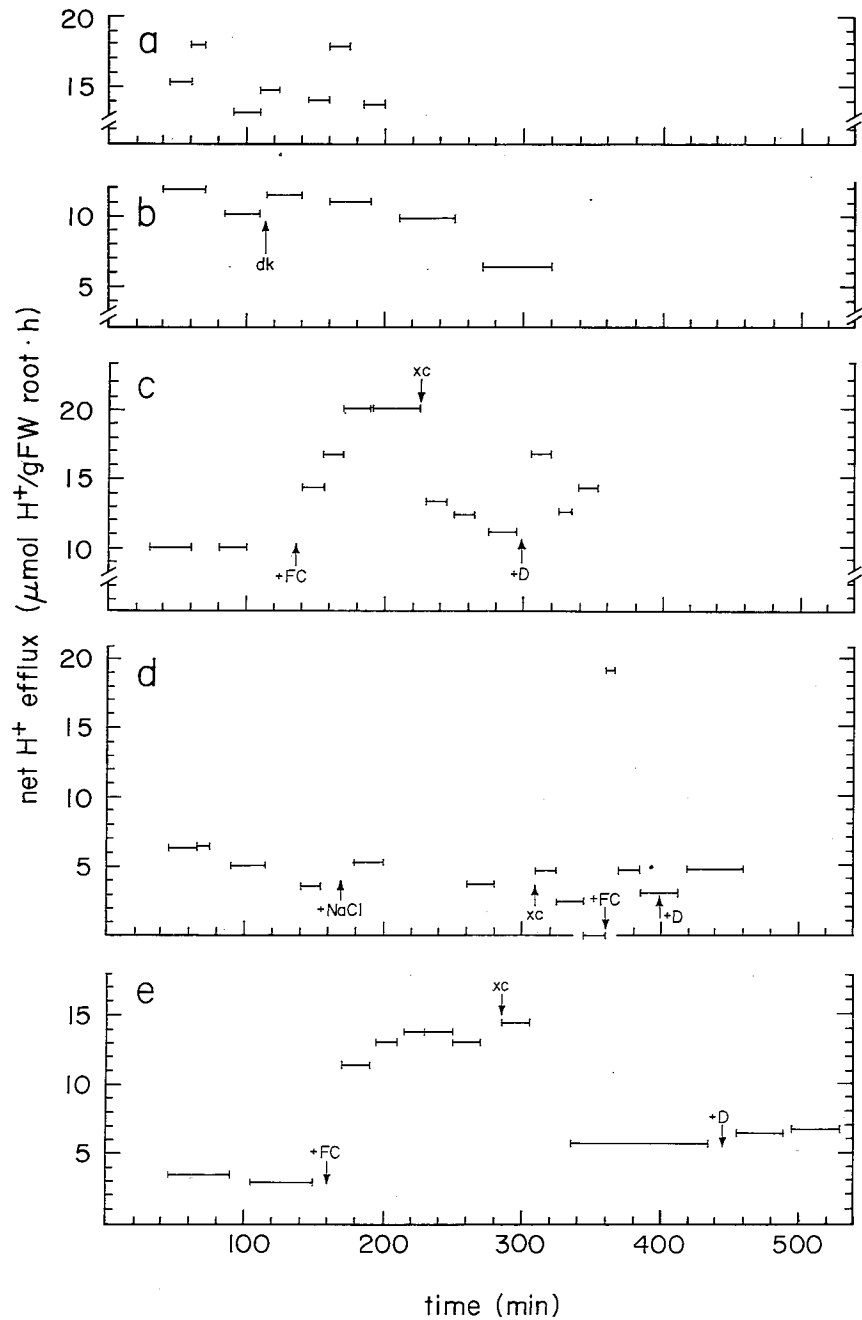


Fig. 1. Net H^+ efflux from roots of *Spergularia marina* ($\mu\text{mol g}^{-1}$ fr. wt. roots h^{-1}). Bars represent linear rates for designated time periods for single experiments. (a) Control: fresh-water grown; 'complete' recording medium. (b) Response of efflux to darkness (dk): fresh-water grown; 'low-salt' medium. (c) Effects of FC (+FC) addition, shoot excision (xc) and addition of dextrose (+D) at points shown: fresh-water grown; 'low-salt' recording medium. (d) Effect of salinity ($45 \text{ mol m}^{-3} \text{ NaCl}$), shoot excision, FC and dextrose: fresh-water grown; 'complete' experimental medium. (e) Response of 0.2x seawater grown plant to FC, excision and dextrose addition; 'low-salt' medium.

DISCUSSION

The net H^+ efflux rates reported here are 2–5 times the rates usually reported for low-salt barley or corn, and are more than 5 times the maximal rates reported for barley or sunflower grown on complete nutrient medium (Bowling *et al.*, 1978; Glass *et al.*, 1981). The response of H^+ efflux to shoot excision and darkness supports the hypothesis that supply of current photosynthate is essential to maintenance of root transport activity (see also Bowling *et al.*, 1978). Figure 1d suggests that FC can cause at least transient diversion of root energy reserves to H^+ transport. External supply of carbohydrate did not completely restore efflux under any conditions.

Despite the general lack of applicable examples, there are numerous considerations of the nature and significance to plant nutrition of the H^+ efflux from roots, the interpretations being classified in two basic groups (Glass and Siddiqi, 1982). In the first class are those reports which stress the importance of cation–anion balances and compensating organic acid production. In the second are reports coupling proton fluxes to K^+ and anion fluxes using modifications of chemi-osmotic theory, the coupling being either chemical or electrical. Glass and Siddiqi (1982) concluded that neither interpretation was sufficient to explain all the experimental results and suggested that an intermediate (or integrated) model would probably prove appropriate.

It is, however, noteworthy that in general such reports are studies of response to nutrient stress, either acknowledged as for iron (Van Egmond and Aktas, 1977) and phosphate (Hedley, Nye, and White, 1983) or not acknowledged (the general case for studies of low-salt seedlings). The present report thus stands as a significant exception.

The theoretical importance of H^+ fluxes in relation to nitrogen acquisition has also been discussed (Raven and Smith, 1976; Smith and Raven, 1979), proton efflux being considered especially important in the case of plants using ammonium as the nitrogen source. There is, so far as we know, only a single study (Becking, 1956) showing the close stoichiometric relationship between ammonium uptake and proton efflux (in *Z. mays* at the age of several weeks, grown on complete nutrient medium). Though a careful study of the relationship between proton fluxes and nitrate assimilation (both in the long and short terms) has been published for the alga *Hydrodictyon africanum* (Raven and De Michelis, 1979), the results are not easily or acceptably extended to higher plants.

At present, data from ion uptake experiments (to be published separately) are insufficient with regard to the nature and significance of H^+ pumping in *S. marina*. The possible relationship to nitrogen metabolism may be considered, as ammonium was present in all complete nutrient media (though not in the low-salt medium). However, nitrate was also present and at higher concentrations, and the ecological literature does not support the hypothesis that ammonium is a preferred nitrogen source for this species (Jefferies, 1977; Jefferies and Perkins, 1977). Thus, although ammonium incorporation may result in excess H^+ production (Smith and Raven, 1979) the current results are not simply and adequately explained on that basis.

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