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Full Length Research Paper

Ionic and water relations of *Sesuvium portulacastrum* (L.)

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The aim of this study was to assess the growth response of *Sesuvium portulacastrum* (L.) (a halophyte) to various NaCl concentrations and hence determine how it is adapted to grow optimally under saline conditions. *S. portulacastrum* was grown hydroponically in various NaCl concentrations (that is, 0, 100, 200, 400 and 600 mol m^{-3} NaCl). It was found to exhibit a growth pattern typical of dicotyledonous halophytes. It attained maximal growth at 200 mol m^{-3} NaCl concentration in hydroponic cultures. It accumulated large concentrations of Na^+ and Cl^- with the leaves having the highest ion content. These ions are believed to be sequestered in the vacuole. *S. portulacastrum* also accumulated proline in high concentrations and this proline appeared to adjust the cytoplasmic ion concentration to balance that of the vacuole.

Key words: *S. portulacastrum*, halophyte, proline, salinity.

INTRODUCTION

The ability to tolerate high external salinities by halophytes depends heavily on osmotic adjustment within the body of plant. This osmotic adjustment appears to be achieved in part by the accumulation of sodium and chloride ions, which often reach concentrations of 500 mol m^{-3} or more (based on the water content) in the leaf tissues (Flowers et al., 1977; Hasegawa et al., 2000).

Associated with this massive inorganic ion accumulation in the vacuole, is the synthesis of compatible organic solutes (that is compatible with cell metabolism), which are believed to adjust the cytoplasmic concentration to balance that of the vacuole (Gorham et al., 1980). Of all organic compounds isolated so far which are believed to act as compatible cytosolutes, proline appears to be the dominant one in many plant species.

According to Venekamp et al. (1989), proline accumulation occurs particularly when water stress coincides with high night temperatures. At night it is thought an active respiration would stimulate synthesis of organic acids, and more glutamate, the main precursor of proline (Singh et al., 1973) would be produced.

In young plants of *Aster trifolium*, the free proline level expressed on a dry matter basis is directly correlated with sodium chloride concentration in the nutrient solution and such a response according to Goas et al. (1982) strongly suggests a function for proline in salt resistance. Equally interesting is the fact that proline increases proportionally faster than other amino acids in plants under water stress and has thus been suggested as an evaluating parameter for irrigation scheduling and for selecting drought resistant varieties (Bates et al. 1973).

According to Greenway (1968), the rate of plant growth under saline conditions is affected by the concentration of salt inside the cells. He further stated that, even though the rate of salt uptake in halophytes is high, the concentration inside their cells remains low, due to dilution. Consequently, growth rates of those plants are not reduced by salinity, unless when growing in excess of 300 mol m^{-3} NaCl (Naidoo and Rughunanan, 1990).

Growth in this respect must be expressed in terms of dry, rather than fresh weight, since the increasing succulence of plants caused by NaCl may result in false interpretation of the effect of salinity (Waisel, 1972).

Halophytes display a totally different mechanism of salt tolerance or regulation. Yeo and Flowers (1980) noted that they amass very high salt concentrations in the leaves. A point worth noting is that the mechanisms which impart salt tolerance to some plants and sensitivity to others have not been resolved (Cheeseman, 1988). Na/K ratios are generally high in halophytes in contrast to glycophytes, although there is a relative enrichment of K^+ in the plant in comparison to the medium (Flowers, 1975).

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It has however, been reported that the amount of K^+ accumulated in some halophytes (namely, *Allenrolfea* and *Sesuvium divaricata*) can exceed the amount of Na^+ (Chapman, 1968). Flowers (1975), however, suggested that such a situation could reflect a low Na^+ content in the growth medium. It should be noted that salinity remains a major threat to sustainable irrigation required to meet the food demands of increasing human population growth (Flowers, 2004).

The aim of the study was to assess growth response of *S. portulacastrum* to elevated concentration of NaCl and inorganic ion distribution of *S. portulacastrum*, when exposed to various NaCl concentrations. The role of proline as a possible osmoticum or compatible cytosolute in *S. portulacastrum* was also investigated.

MATERIALS AND METHODS

Cultivation

Stem cuttings 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, unless otherwise stated, plant cuttings were taken from the stock plants, allowed to root (a rooting hormone was applied to the cut end) for two weeks in vermiculate 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 Peterson (1984) culture solution. The plants were then transferred into the 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^{-3} NaCl for two and a half months. The plants were grown at 24°C under continuous light from warm-white fluorescent tubes giving a mean light intensity of 206 µmoles 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 the plants. The concentration was increased by 100 mol m^{-3}, 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 before determining the fresh weight. Having determined the fresh weight, 1.0 g of each from the root tissue and shoot tissue was cut for the analysis of inorganic ions. The rest of shoot and root tissues were dried in an oven at 80°C for 48 h for dry weight determination.

Tissue ion concentrations

Roots and leaves were separately boiled in 25 ml of distilled water for 20 min to extract sap. The extract was filtered through Whatman (grade 1) filter paper. The filtrate was made up to 100 ml with distilled water. Concentrations of K^+ and Na^+ were determined with a flame photometer (JENWAY PFP 1). Cl^- concentration was determined with a chloride selective electrode. The ion content of leaves and stem was analysed as described above. For calculation of ion concentration in the plant tissue, it was assumed tha t 1 g of fresh weight tissue is equivalent to 1 cm^{-3} of water. After determining the density of the different plant tissues (roots, stem, leaves and shoots), the ion concentration was adjusted accordingly.

Determination of proline in leaves and roots

Plants were grown hydroponically in culture solution containing various concentrations of NaCl (that is 0, 100, 200, 400 and 600 mol m^{-3} NaCl). Proline analysis was carried out according to Bates et al. (1973) method. Plant tissue (either root or leaf) were sampled as follows: approximately 0.1 g of plant tissue was homogenized in 10 ml of 3% aqueous sulfosalicyclic acid and the homogenate filtered through Whatman filter (grade 1) paper. Two ml of the filtrate was reacted with two ml of acid ninhydrin (prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 Molar phosphoric acid, with agitation, until dissolved) and two ml of glacial acetic acid in a test tube for one hour at 100°C, and the reaction terminated in an ice bath.

The reaction mixture was extracted with 4 ml of toluene, mixed vigorously with a test tube stirrer. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm on a SPEKOL spectrocolorimeter. Toluene was used for a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows: [(µg proline/ml x ml toluene)/115.5 µg/pmole]/ [(g sample)/ 5] = µmole proline/g of fresh weight material.

Determination of tissue water potential

The experiment was performed using plants grown hydroponically for one month at different NaCl concentrations (0, 100, 200, 400, and 600 mol m^{-3}). For determination of tissue water potentials, leaves were harvested and washed in distilled water to remove any spilled salts and then blotted dry. Using a cork borer two leaf segments were cut from different leaves and placed in a Wescor C-51 Sample Chamber Psychrometer, WESCOR, INC. The C-51 Sample Chamber Psychrometer was connected to the HR-33 Dew Point Microvoltmeter which is basically a readout device. It gives readings in microvolts proportional to the amount of solute in solution. Roots were made into a bundle and treated in a similar manner. The root samples were allowed to equilibrate for 10 min, whereas leaf samples were left for 15 - 20 min. These equilibration times were experimentally determined beforehand.

Determination of osmotic potential of tissues

The experiment was carried out on plants grown as described above. Leaves and roots were separately frozen with liquid nitrogen and then pressed onto a filter paper disc (obtained with a standard 1/4" paper punch) until the disc was saturated. The paper disc was then inserted into a C-51 chamber and osmotic potential measured as described above. Equilibration times were determined prior to the experiment and found to be 8 - 10 min. NaCl solutions of known concentrations and osmotic potentials were used for the calibration curve.

Determination of turgor pressure

Since turgor pressure is difficult to measure experimentally, it was determined by taking the difference between osmotic potential and water potential. It was calculated as follows: turgor pressure = osmotic potential - water potential.

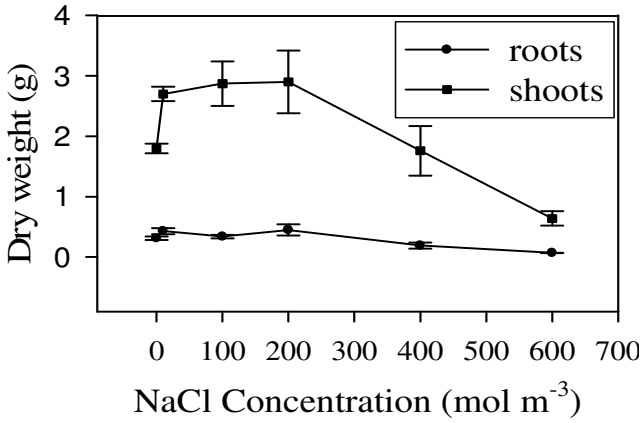

Figure 1. The effect of increasing NaCl concentration of the culture medium on the dry weight of shoots (●) and roots (●) of *S. portulacastrum*. Bars represent S.E.M (n = 4).

RESULTS

Growth response to salinity

Figure 1 shows the time course of dry weight production by *S. portulacastrum*. There was a considerable enhancement of dry mass accumulation with the increase in salinity from 0 to 200 mol m^{-3} NaCl. Maximal dry weight production occurred at 100 and 200 mol m^{-3} NaCl. The enhancement of dry weight did not occur uniformly throughout the plant, but predominantly in the shoots (that is resource shift from roots to shoots as NaCl concentration increased). However, the roots maintained more or less the same weight up to 200 mol m^{-3} NaCl. In general it can be said that in plants originally grown in the absence of NaCl and transferred to various NaCl concentrations, the growth was enhanced in growth medium containing up to 200 mol m^{-3} NaCl. Any increase in NaCl concentration above 200 mol m^{-3} appeared to retard growth. It can be seen that even at 600 mol m^{-3} NaCl, this species still managed to survive, though growth was substantially reduced. In fact, 900 mol m^{-3} NaCl was the concentration which proved lethal to this species (results not shown).

Inorganic ion content

Na^+ and Cl^- concentrations in roots appeared to increase with increasing NaCl concentration in the growth medium (Figure 2). However, the internal ion concentration balanced that of the growth medium up to 200 mol m^{-3} NaCl. K^+ concentration in roots, on the other hand, appeared to be unaffected by the increase in NaCl concentration up to 400 mol m^{-3} NaCl. There was a decline in K^+ and Na^+ content in the roots at salinities above 40 mol m^- NaCl. Unlike the roots, Na^+ and Cl^- in the shoots almost balanced that of the culture solution at high external concentrations. It appeared that the concentration of Na^+ and Cl^- is higher in the shoot than in the roots (Figure 2).

At 0 mol m^{-3} NaCl, the total Na^+ content in the roots was about 0.043 moles, while that in the shoot was 0.339 moles. At 200 mol m^{-3} NaCl the Na^+ content was 1.000 and 0.527 moles, in the shoot and roots, respectively.

This shows that most of the Na^+ was in the shoot. There was a high K^+ content in the shoots at lower salinities. However, as the NaCl concentration of the culture solution increased, there was a marked decrease in K^+ concentration in the shoots (Figure 2).

It was evident that the leaves accumulated more proline than did the roots (Figure 3). At 200 mol m^{-3} NaCl of the external medium, there was a drop in proline concentration in the roots. This drop in proline content in the roots was associated with a high praline content in the leaves. A point of considerable interest is that proline content increased with increasing NaCl concentration of the growth medium. However, at higher salinities (that is above 400 mol m^{-3} NaCl) there was a marked decline in proline content in the leaves.

The relationship between osmotic potential of the external solution and cell water potential, cell osmotic potential and turgor potential of root and leaf tissues are presented in Figure 4. Both water potential and solute (osmotic) potentials of leaves were substantially lower than those of the roots. Infact more negative osmotic values of about -4.1 MPa in leaves were recorded at higher concentrations of NaCl in the external medium. As the NaCl concentration of the culture solution increased to 600 mol m^{-3}, the osmotic potential of the roots became less negative. Turgor pressure of the roots approached 1 MPa in plants grown in culture solutions of higher NaCl concentrations (Figure 4). Turgor pressure of the leaves, on the other, appeared to slightly decrease with increasing NaCl concentration of the culture solution (Figure 4).

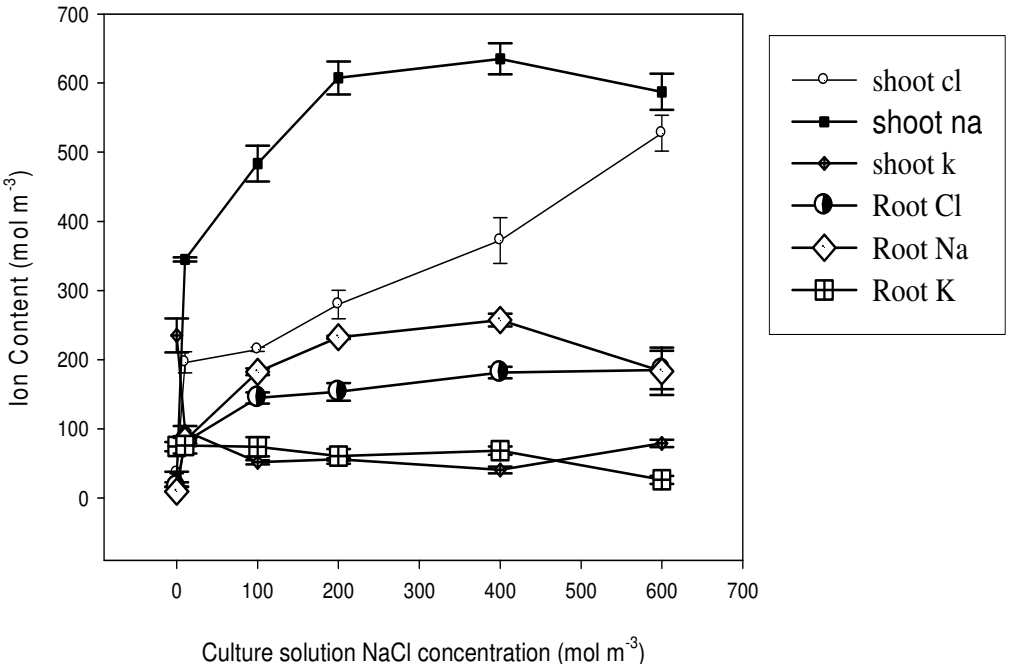

Figure 2. The effect of increasing NaCl concentration on ionic distribution in the roots the shoots of *S. portulacastrum*. Bars represent S.E.M (n = 4).

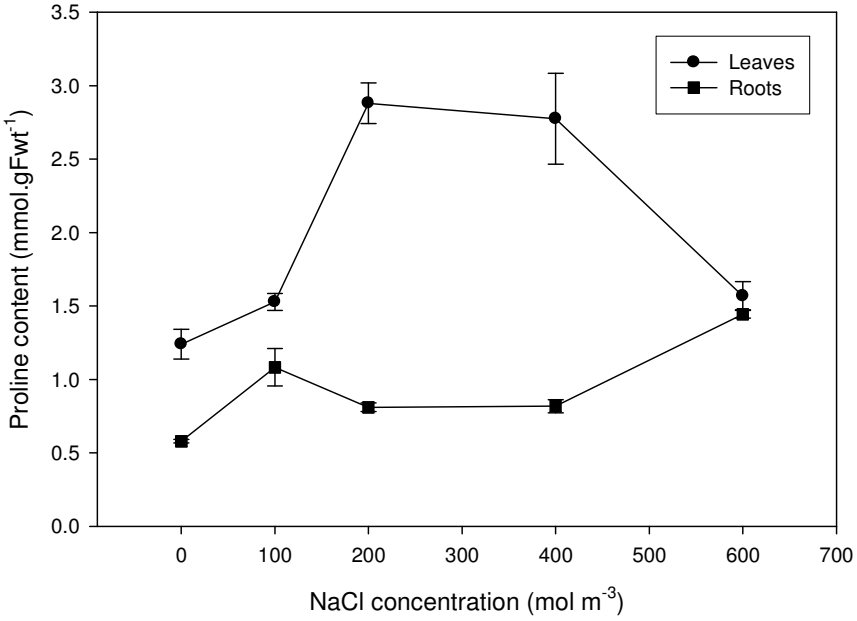

Figure 3. The effect of increasing NaCl concentration of the culture medium on proline content of leaves and roots of *Sesuvium portulacastrum*. Bars represent S.E.M (n = 4).

DISCUSSION

Dry mass production increased with the increase in salinity up to 200 mol m^{-3} NaCl. This shows that *S. portulacastrum* is highly salt tolerant and exhibited an extended growth response to NaCl (Figure 1). This is in agreement with previous reports that halophytes, in general, exhibit growth optima at 200 mol m^{-3} NaCl (Munns et al., 1983; Flowers et al., 1986). There is evidence to suggest that some halophytes displayed growth optimum at 300 mol m^{-3} NaCl (Naidoo and Rughunanan, 1990). It is important, therefore, to note that

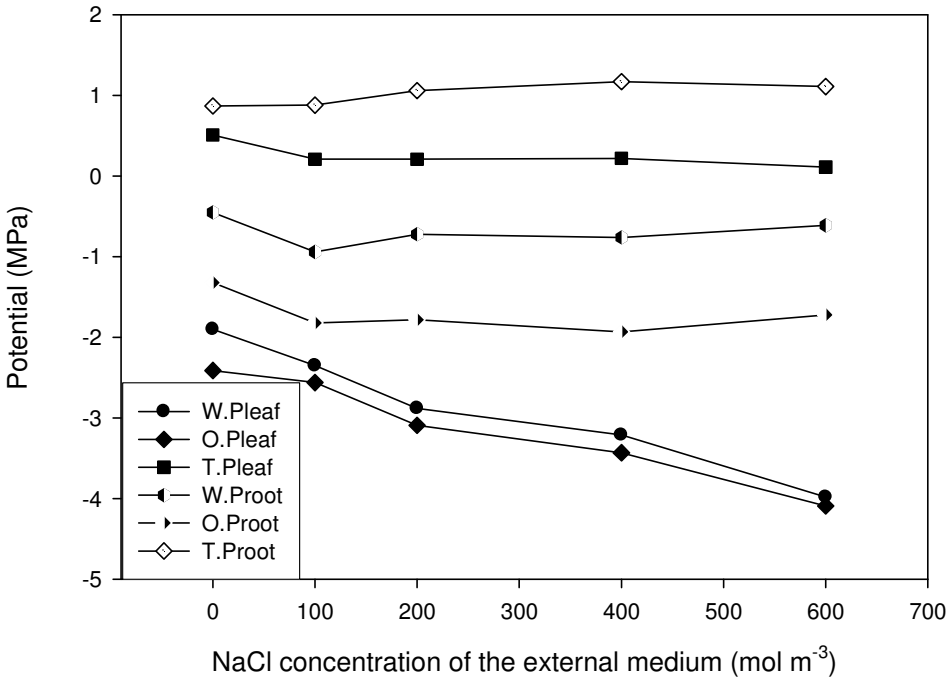

Figure 4. The effect of increasing NaCl concentration of the external solution on cell water potential (W.P), osmotic potential (O.P) and turgor pressure (T.P) of leaf and root tissues of *S. portulacastrum*.

halophytes even though salt tolerant, do vary with respect to the optimal NaCl concentration that gives maximum dry weight. Flowers (1975) pointed out that optima are often imprecisely known, as for example, different concentrations of NaCl were employed in this study.

It is most probable that in addition to massive ion uptake, the expansive growth as Naidoo and Rughunanan (1990) suggested, may be associated with adequate supply of carbon resources. The reduction in plant dry mass at salinities above 200 mol m^{-3} NaCl (Figure 1) might be ascribed to the re-allocation of carbon resources from cumulative growth to the production of Osmolytes like proline (Figure 3) for osmotic adjustment and cellular maintenance. In fact, it has been previously shown that high salinities do, in effect, reduce carbon acquisition (Robinson et al., 1983), thus leading to reduction in growth and dry mass accumulation (Figure 1). Similar results have been obtained by Cramer (2003) who demonstrated that salinity inhibited leaf elongation rates of three different grass species.

S. portulacastrum exhibited a typical inorganic ion distribution common to other halophytes, in which the major portion of the absorbed Na^+ and Cl^- is transferred to the shoot (Flowers et al., 1977). This results in reducing K^+ concentration especially in the leaves (Figure 2). The effects of Na^+ on reducing K^+ is well documented (Cramer et al., 1985; Clipson and Flowers, 1987) and may possibly limit growth at high salinities (Flowers and Lauchli, 1983). It is logical to suggest here that most of the Na^+ which accumulated in the leaves is compartmentalized in the vacuole so as to exclude it from the cytoplasm (Flowers et al., 1977). Flowers and Yeo (1986) argued that if the volume of the cytoplasm (5 - 10% of cell volume) and that of the vacuole (about 90% of cell volume) is taken into account as a storage organ, then, it is logical to suggest the vacuole as the main storage organ of the massive inorganic ion content in halophytes. The high proline content (Figure 3) presumably adjusts the cytoplasmic water potential to balance that of the vacuole, in which most of the inorganic ions were sequestered as has been previously suggested by Flowers et al. (1986).

Both osmotic and water potential values (Figure 4) of leaves were maintained more negative than that of the roots. These low values (osmotic and water potential) ensured influx of water into the shoot. Similar results were obtained by Rhizopoulou and Mitrakos (1990), working on water relations of evergreen sclerophyll of the mediterranean. They reported leaf water potential and osmotic potential of -3.5 MPa and -4.0 to - 4.5 MPa, respectively. It is suggested that the low osmotic potential values in the leaves are due to massive accumulation of inorganic ions (Na^+ and Cl^- in particular) (Figure 2) and proline (Figure3) as a compatible solute.

S. portulacastrum (L.) was found to exhibit a growth pattern typical of dicotyledonous halophytes. It attained maximal growth at 200 mol m^{-3} NaCl concentration in hydroponic cultures. It accumulated large concentrations

of Na^+ and Cl^- with the leaves having the highest ion content. These ions are believed to be sequestered in the vacuole. *S. portulacastrum* (L.) also accumulated proline in high concentrations, and this proline appeared to adjust the cytoplasmic ion concentration to balance that of the vacuole.

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