Acta Bot. Croat. 60 (2), 237–244, 2001

CODEN: ABCRA 25
ISSN 0365–0588
UDC 581.18

Dedicated to Prof. dr. sc. ZVONIMIR DEVIDÉ on the occasion of his 80th birthday

The effect of salinity and osmotic stress on duckweed *Lemna minor* L.

MIRTA TKALEC*, JELENA MLINAREC, ŽELJKA VIDAKOVIĆ-CIFREK, BISERKA JELENČIĆ, IVAN REGULA

University of Zagreb, Faculty of Science, Department of Botany, Rooseveltov trg 6, HR-10000 Zagreb, Croatia

The response of duckweed (*Lemna minor* L.) to salinity and osmotic stress was evaluated by monitoring growth and peroxidase activity every second day, for a period of 17 days. The nutrient medium was supplemented with isoosmolar concentrations of NaCl and mannitol (50 mM and 100 mM NaCl, 100 mM and 200 mM mannitol). Growth decreased markedly with increasing concentrations of NaCl and mannitol. The lower concentrations of NaCl (50 mM) and mannitol (100 mM) started to reduce growth significantly in the second week of the experiment, while the higher concentrations (100 and 200 mM) reduced growth from the beginning. Protein concentrations and peroxidase activity fluctuated during the growth period but, most of the time, they were increased in plants treated with NaCl and mannitol. These results suggest that *Lemna minor* is sensitive to both salinity and osmotic stress. However, the continued growth, and also the increase in peroxidase activity and protein concentration, during the NaCl and mannitol treatments, demonstrate its potential for adaptation to long-term stress. Although salinity and osmotic stress have similar overall effects on *Lemna minor*, consistent minor differences in growth, protein concentration and peroxidase activity between plants grown on NaCl and those grown on mannitol suggest overlapping, rather than identical, mechanisms of adaptation to salinity and osmotic stress.

Key words: *Lemna minor*, salinity, osmotic stress, sodium chloride, mannitol

Introduction

Salinity stress is a widespread environmental problem. Approximately 50 % of the irrigated land is affected by high salinity (LAUCHLI 1991, cit. by KNIGHT et al. 1997) which includes Na^+ and Cl^- as the most common ions (MUNNS and TERMAAT 1986). Most plants are sensitive to salinity above 50–100 mM NaCl while some halophytic species can tolerate 500 mM NaCl (DOWNTON 1984). Salinity imposes two types of stress, both of which can severely affect plant growth (TAIZ and ZEIGER 1991). The first is osmotic stress caused by

* Corresponding author: Fax: 385 1 48 26 260, E-mail: mtkalec@zg.biol.pmf.hr

high ion concentrations in the growth medium which decrease its water potential, and it is more difficult for the plant to absorb water and nutrients. The second is specific toxicity of ions present in high concentrations, which can interfere with the uptake of nutrients, cause membrane damage and directly inhibit cellular metabolism (KNIGHT et al. 1997, VOLKMAR et al. 1998). Plants respond to salinity by osmotic adjustment, usually by increasing the concentrations of inorganic ions or organic solutes, such as prolin, in their cells and vacuoles (HERNÁNDEZ et al. 1999, SAVOURÉ et al. 1999). Salinity also causes oxidative stress and thus triggers defence mechanisms targeting harmful reactive oxygen intermediates. In case of NaCl stress, for instance, plants respond with increased activity of antioxidative enzymes (GOSSETT et al. 1994, HERNÁNDEZ et al. 1999).

Duckweed (*Lemna minor*) is a small, aquatic, floating monocot adapted to freshwater habitats that grows rapidly and mostly reproduces vegetatively. Easiness of culture and sensitivity to a wide range of toxic substances, such as pesticides and heavy metals, make this plant a suitable test organism (LEWIS 1995).

Duckweeds are considered to be salt-sensitive (HILLMAN 1961). To find out if this is due to the osmotic effect of salt or to specific ion toxicity, the effect of isoosmolar concentrations of NaCl and mannitol on the growth of *Lemna minor* was investigated. We also studied the activity of peroxidase, an antioxidative enzyme involved in detoxification, to establish possible correlations between the activity of this enzyme and the sensitivity of duckweed to salinity stress.

Materials and methods

Plant material and culture

Lemna minor L. was originally collected in the Botanical Garden of the Faculty of Science, University of Zagreb. Plants were sterilised according to KRAJNČIČ and DEVIDÉ (1980) and maintained as stock cultures under axenic conditions on Pirson-Seidel's nutrient solution (PIRSON and SEIDEL 1950).

For both, stock and experimental cultures, the pH value of the nutrient solution was adjusted to 4.55 with 0.1 M KOH and autoclaved at 0.15 MPa and 120 °C for 20 minutes. The cultures were grown under 16 hours of light (80 $\mu Em^{-2}s^{-1}$) at 24±2 °C.

Plant growth

Growth experiments were started by transferring single healthy colonies with 2–3 fronds from stock cultures into 100 ml Erlenmeyer flasks containing 60 ml of Pirson-Seidel's nutrient solution supplemented with the quantities of NaCl (Kemika) or mannitol (Sigma) necessary to afford concentrations of 50 mM or 100 mM NaCl and 100 mM or 200 mM mannitol. Each treatment and the control were prepared in eight replicates.

Frond numbers were monitored during a 17-day-period, on days 0, 3, 5, 8, 10, 12, 15 and 17. Growth (G) was expressed in terms of relative frond numbers which were calculated using the expression (ENSLEY et al. 1994):

$$G = \frac{\text{no. of fronds at day n} - \text{no. of fronds at day 0}}{\text{no. of fronds at day 0}}, \qquad n = 3, 5, 8, 10, 12, 15, 17$$

The results are given as means of eight replicates ± standard error of the mean and tested for significance using Duncan's New Multiple Range Test (DUNCAN 1955).

Peroxidase activity

In this set of experiments, cultures were started by transferring 5–10 healthy colonies with 2–3 fronds from stock cultures into 250 ml Erlenmeyer flasks containing 100 ml of Pirson-Seidel's nutrient solution supplemented with NaCl or mannitol in the same concentrations as in the growth experiments.

Every second days, during a 17-day-period, aliquots of plant material were collected axenically from each treatment and the control and worked up as follows. For measuring protein content, 50 mg of plant material were homogenised in 1.5 ml of buffer prepared according to STAPLES and STAHMANN (1964) with the addition of polyvinylpolypyrrolidone (Sigma). For measuring peroxidase activity, 100 mg of plant material were homogenised in 1.0 ml of KCl/K_3PO_4 buffer, pH 7.0 (PETERS et al. 1988) with the addition of polyvinylpolypyrrolidone. Both homogenates were centrifuged at 18000*g* at 4 °C for 60 minutes and the supernatants were used for analysis.

Soluble protein content was estimated by the method of Bradford (BRADFORD 1976) using bovine serum albumin as a standard, and expressed as the mean of two experiments.

Peroxidase activity was determined spectrophotometrically at 470 nm using the guaiacol oxidation method (MÄDER et al. 1975). The reaction mixture was prepared according to SIEGEL and GALSTON (1967). Peroxidase activity was expressed on a fresh mass basis, as the mean of two experiments.

Results

The osmotic and salt stress applied did not cause obvious symptoms of toxicity, such as chlorosis or reduction in frond size. The relative frond number of *Lemna minor* did, however, decrease significantly with increasing concentrations of NaCl and mannitol (Fig. 1). Comparing the growth under both applied concentrations of NaCl and mannitol, there was no statistically significant difference between the effect of these two substances. At the lower concentrations of NaCl (50 mM) and mannitol (100 mM), growth was slightly reduced during the first week, but was not significantly different from that in the control. Then, from day 8 for NaCl treatment, and day 10 for mannitol treatment, until the end of growth period, growth was reduced significantly ($P = 0.05$) in comparison with the control (Fig. 1A). In contrast, at higher concentrations, both NaCl (100 mM) and mannitol (200 mM) significantly ($P = 0.05$) reduced growth during the entire period of exposure (Fig. 1B).

The controls as well as all treatments showed an initial increase of protein concentration, and then maintained the respective protein levels until the end of the experiment. These levels were generally higher under osmotic and salt stress than in the controls (Fig. 2A).

Guaiacol peroxidase activity, expressed on a fresh matter basis, in all treatments and the control showed similar values during the first week of the experiment, while, during the

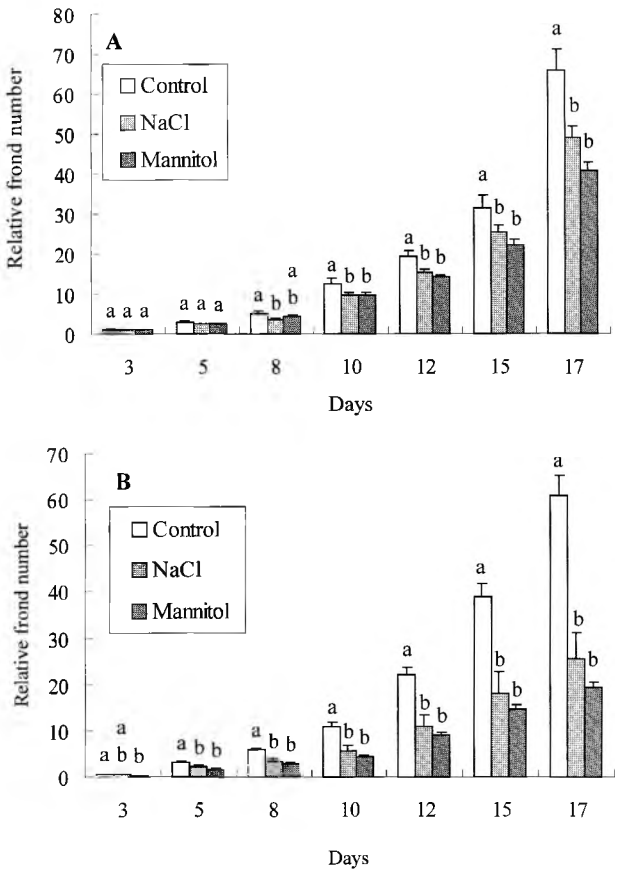

Fig. 1. *Lemna minor* L. growth expressed as relative frond number (see Materials and Methods for definition) during a growth period of 17 days in nutrient solutions containing A) 50 mM NaCl or 100 mM mannitol and B) 100 mM NaCl or 200 mM mannitol. Each value is the mean of eight replicates ± standard error. Bars indicate standard errors. Different letters on the top of the columns indicate significant differences between treatments, monitored on the same day at P = 0.05 by Duncan's New Multiple Range Test.

rest of growth period, osmotic as well as salt stress caused an increase of peroxidase activity (Fig. 2B). Similar results were obtained in two independent experiments.

Discussion

Although duckweed *Lemna minor* L. is considered salt-sensitive (HILLMAN 1961), long-term (17 days) osmotic and salt stress did not cause obvious symptoms of toxicity or nutrient disbalance, such as chlorosis, and the plants were still able to grow. However, higher concentrations of NaCl (100 mM) and mannitol (200 mM) caused strong growth reduction during the whole growth period (Fig. 1B). It seems that duckweed can adapt to some extent to osmotic and salt stress and survive, but at the expense of growth reduction.

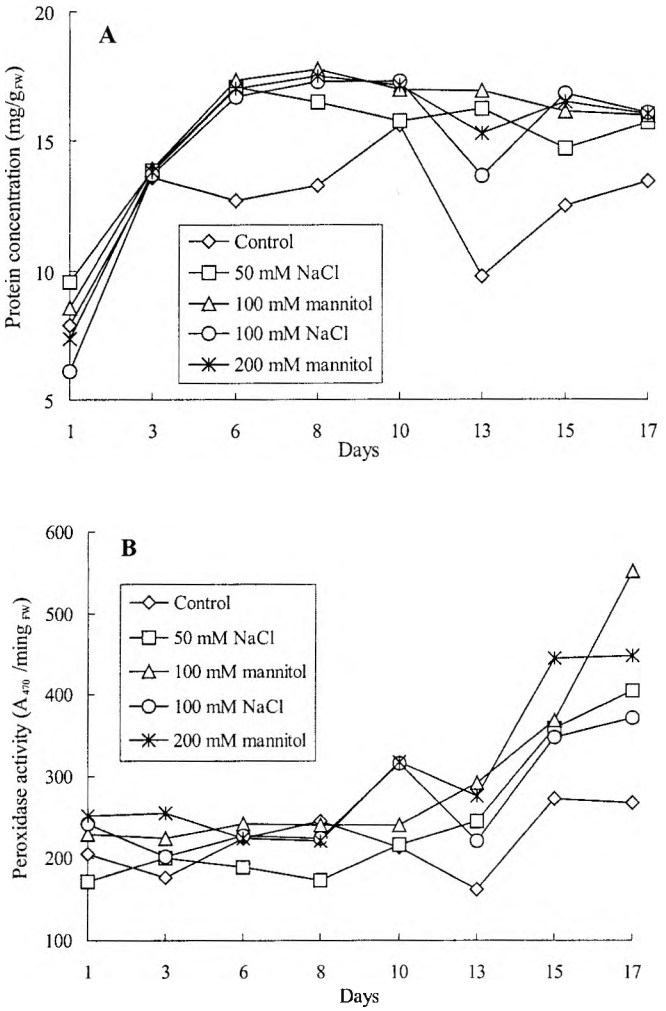

Fig. 2. Effect of NaCl and mannitol during a growth period of 17 days on A) protein concentration and B) guaiacol peroxidase activity expressed on a fresh matter basis. Each value is the mean of two independent experiments.

Our results showed that there was no significant difference (P = 0.05) between the effects of NaCl and mannitol on growth. FRICK and GOLT (1995) obtained similar results with NaCl and mannitol and also noticed that, after treatment with Na_2SO_4, plants accumulated large amounts of SO_4^{2-} ions, but not of Na^+ ions. This suggests that *Lemna minor* is sensitive to Na^+ (and possibly Cl^-) ions and thus developed efficient mechanisms for avoiding their accumulation. Yet, mannitol reduced growth to a slightly greater extent than NaCl (Fig 1.) which could mean that Na^+ ions, at least to some degree, serve for osmotic adjustment. It has already been reported that the accumulation of NaCl permits osmotic adjustment without using metabolic energy and is thus widely used by plants tolerant to salt

(halophytes). Plants sensitive to salt usually accumulate organic solutes, a process which consumes assimilates and thus tends to lead to reduced growth (PÉREZ-ALFOCEA et al. 1993, EGAN and UNGAR 1998).

It has been suggested that peroxidase activity increases in response to stress conditions because the enzyme is involved in detoxification of the H_2O_2 over-produced during oxidative stress (GUETA-DAHAN et al. 1997, HERNÁNDEZ et al. 1999). The present work showed an increase of peroxidase activity in response to osmotic and salt stress (Fig. 2B). These results corroborate published reports (GOSSETT et al. 1994, HERNÁNDEZ et al. 1999) stating that osmotic and salt stress are accompanied by oxidative stress. It also appears that this oxidative stress is due to osmotic effects and not to specific ion effects because there were no major differences in peroxidase activity between NaCl and mannitol stress. LECHNO et al. (1997) found that NaCl and KCl in cucumber plants increased the activities of catalase and glutathione reductase which are also antioxidative enzymes involved in H_2O_2 detoxification and also suggested a correlation with the osmotic effect of salts. However, the fluctuations of peroxidase activity we observed during the growth period under the influence of NaCl and mannitol treatments suggest complex functions of this enzyme in plants during their growth and perhaps different ways of activation.

Soluble protein concentrations also fluctuated during the growth period but most of the time they were increased with both concentrations of NaCl and mannitol (Fig. 2A). It would be interesting to investigate if this increase is due to the synthesis of specific stress proteins included in osmotic adjustment. It has already been reported that NaCl induced the accumulation of five polypeptides in *Mesembryanthemum crystallinum* (YEN et al. 1997). There may be a connection between growth reduction and the synthesis of stress proteins, in which case the sensitivity of *Lemna minor* to osmotic and salt stress would be due to spending lots of energy for osmotic adjustment.

The results presented here confirm that *Lemna minor* is sensitive to salinity and osmotic stress, but also has the ability for adaptation because it continued to grow in spite of long-term exposure to NaCl and mannitol. The increase of peroxidase activity in the treated plants shows that osmotic and salt stress is accompanied by oxidative stress and that duckweed has the capacity to cope with it. Although we did not find significant differences between salinity and osmotic effects, in the range of concentrations applied, consistent minor differences in growth, protein concentrations and peroxidase activity between plants treated with NaCl and mannitol suggest that the mechanisms of long-term adaptation to salt and osmotic stress may not be completely identical.

References

BRADFORD, M.M., 1976: A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

DOWNTON, W.J.S., 1984: Salt tolerance of food crops: Perspectives for improvements. Crit. Rev. Plant. Sci 1, 183–201.

DUNCAN, D.B., 1955: Multiple range and multiple F tests. Biometrics 11, 1–42.

EGAN, T.P., UNGAR, I.A., 1998: Effect of different salts of sodium and potassium on the growth of *Atriplex prostrata* (*Chenopodiaceae*). J. Plant Nutr. 20, 1723–1730.

ENSLEY, H.E., BARBER, J.T., POLITO, M.A., OLIVER, A.I., 1994: Toxicity and metabolism of 2,4-dichlorophenol by the aquatic angiosperm *Lemna gibba*. Environ. Toxicol. Chem. 13(2), 325–331.

FRICK, H., GOLT, C., 1995: Sensitivity of *Lemna minor* growth to osmotic potential and relative tolerance of its callus. J. Plant Physiol. 146, 718–724.

GOSSETT, D.R., MILLHOLLON, E.P., LUCAS, M.C., BANKS, S.W., MARNEY, M.M., 1994: The effect of NaCl on antioxidant enzyme activities in callus tissue of salt-tolerant and salt-sensitive cotton cultivars (*Gossypium hirsutum* L.). Plant Cell Rep. 13, 498–503.

GUETA-DAHAN, Y., YANIV, Z., ZILINSKAS, B.A., BEN-HAYYIM, G., 1997: Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in *Citrus*. Planta 203, 460–469.

HERNÁNDEZ, J.A., CAMPILLO, A., JIMÉNEZ, A., ALARCÓN, J.J., SEVILLA, F., 1999: Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. New Phytol. 141, 241–251.

HILLMAN, W.S., 1961: The *Lemnaceae* or duckweeds. Bot. Rev. 27, 221–287.

KNIGHT, H., TREWAVAS, A.J., KNIGHT, M.R., 1997: Calcium signalling in *Arabidopsis thaliana* responding to drought and salinity. Plant J. 12(5), 1067–1078.

KRAJNČIČ, B., DEVIDÉ, Z., 1980: Report on photoperiodic responses in *Lemnaceae* from Slovenia. Ber. Geobot. Inst. 47, 75–86.

LECHNO, S., ZAMSKI, E., TEL-OR, E., 1997: Salt stress-induced responses in cucumber plants. J. Plant Physiol. 150, 206–211.

LEWIS, M.A., 1995: Use of freshwater plants for phytotoxicity testing: a review. Environ. Pollut. 87, 319–336.

MÄDER, M., MÜNCH, P., BOOP, M., 1975: Regulation und Bedeutung der Peroxidase-Musteränderungen in sprossdifferenzierenden Kallus Kulturen von *Nicotiana tabacum* L. Planta 123, 257–265.

MUNNS, R., TERMAAT, A., 1986: Whole-plant response to salinity. Aust. J. Plant Physiol. 13, 143–160.

PÉREZ-ALFOCEA, F., ESTAÑ, M.T., CARO, M., GUERRIER, G., 1993: Osmotic adjustment in *Lycopersicon esculentum* and *L. pennellii* under NaCl and polyethylene glycol 6000 iso-osmotic stresses. Physiol. Plant. 87, 493–498.

PETERS, J.L., CASTILLO, F.J., HEATH, R.L, 1988: Alteration of extracellular enzymes in pinto bean leaves upon exposure to air pollutants, ozone and sulphur dioxide. Plant Physiol. 89, 159–164.

PIRSON, A., SEIDEL, F., 1950: Zell- und stoffwechselphysiologiche Untersuchungen an der Wurzel von *Lemna minor* unter besonderer Berücksichtigung von Kalium- und Calciummangel. Planta 38, 431–473.

SAVOURÉ, A., THORIN, D., DAVEY, M., HUA, X.J., MAURO, S., VAN MONTAGU, M., INZÉ, VERBRUGGEN, N., 1999: NaCl and $CuSO_4$ treatments trigger distinct oxidative defence mechanisms in *Nicotiana plumbaginifolia* L. Plant Cell Environ. 22, 387–396.

SIEGEL, B.Z., GALSTON, W., 1967: The peroxidase of *Pisum sativum*. Plant Physiol. 42, 221–226.

STAPLES, R.C., STAHMANN, M.A., 1964: Changes in proteins and several enzymes in susceptible bean leaves after infection by the bean rust fungus. Phytopatology 54, 760–764.

TAIZ, L., ZEIGER, E., 1991: Plant physiology. The Benjamin/Cummings Publishing Company, Inc., Redwood City.

VOLKMAR, K.M., HU, Y., STEPPUHN, H., 1998: Physiological responses of plants to salinity: A review. Can. J. Plant Sci. 78, 19–27.

YEN, H.E., ZHANG, D., LIN, J.-H., EDWARDS, G.E., KU, M.S.B., 1997: Salt-induced changes in protein composition in light-grown callus of *Mesembryanthemum crystallinum*. Physiol. Plant. 101, 526–532.