PHYSIOLOGIA PLANTARUM 84: 600–605. Copenhagen 1992

# Supplemental manganese improves the relative growth, net assimilation and photosynthetic rates of salt-stressed barley

Grant R. Cramer and Robert S. Nowak

Cramer, G. R. and Nowak, R. S. 1992. Supplemental manganese improves the relative growth, net assimilation and photosynthetic rates of salt-stressed barley. – Physiol. Plant. 84: 600–605.

Previous results in our laboratory indicated that a reduced Mn concentration in the leaves of barley was highly correlated with the reduced relative growth and net assimilation rates of salt-stressed plants. If Mn deficiency limits the growth of salt-stressed barley, then increasing leaf Mn concentrations should increase growth. In the present study, the effect of supplemental Mn on the growth of salt-stressed barley (*Hordeum vulgare* L. cv. CM 72) was tested to determine if a salinity-induced Mn deficiency was limiting growth. Plants were salinized with 125 mol m$^{-3}$ NaCl and 9.6 mol m$^{-3}$ $CaCl_2$. Supplemental Mn was applied in 2 ways: 1) by increasing the Mn concentration in the solution culture and 2) by spraying Mn solutions directly onto the leaves. Growth was markedly inhibited at this salinity level. Dry matter production was increased 100% in salt-stressed plants treated with supplemental Mn to about 32% of the level of nonsalinized controls. The optimum solution culture concentration was 2.0 mmol m$^{-3}$, and the optimum concentration applied to the leaves was 5.0 mol m$^{-3}$. Supplemental Mn did not affect the growth of control plants. Further experiments showed that supplemental Mn increased Mn concentrations and uptake to the shoot. Supplemental Mn increased the relative growth rate of salt-stressed plants and this increase was attributed to an increase in the net assimilation rate; there were no significant effects on the leaf area ratio. Supplemental Mn also increased the net photosynthetic rate of salt-stressed plants. The data support the hypothesis that salinity induced a Mn deficiency in the shoot, which partially reduced photosynthetic rates and growth.

*Key words* – Barley, growth analysis, *Hordeum vulgare*, leaf area ratio, Mn, net assimilation rate, photosynthesis, relative growth rate, salinity, uptake.

*G. R. Cramer (corresponding author), Dept of Biochemistry, Univ. of Nevada, Reno, NV 89557, USA; R. S. Nowak, Dept of Range, Wildlife and Forestry, Univ. of Nevada, Reno, NV 89557, USA.*

## Introduction

Salinity inhibits the growth of many plants (Greenway and Munns 1980, Munns and Termaat 1986). Salinity appears to affect two plant processes: water and ionic relations. During the initial exposure to salinity, the plant experiences water stress, which in turn reduces leaf expansion (Munns and Termaat 1986). In addition, during long-term exposure to salinity, the plant experiences ionic stress which can lead to premature senescence of adult leaves, and thus a reduction in the photosynthetic area available to support continued growth.

There has been an extensive amount of research on the effects of salinity on barley (to our knowledge at least 90 publications have appeared). In the early 1960's, Greenway (1962a, b, 1963, 1965) showed that the difference in the salt tolerance of barley cultivars was related to their ability to regulate ion transport. In particular, Na exclusion seemed to be important (Greenway 1962a). Twenty years later, Munns et al. (1982) assessed the contribution of the osmotic effects of salinity relative to its ionic effects and concluded that the inhibitory effects on growth were mostly due to osmotic and not ion-specific effects. This conclusion is supported by other reports (e.g. Termaat and Munns 1986, Thiel et al. 1988).

---

Received 4 September, 1991; revised 2 December, 1991

However, in addition to the osmotic effects, ion-specific effects can have a significant impact on the growth of salt-stressed barley. This has been demonstrated in recent studies on barley (e.g. Cramer et al. 1989, 1990, 1991, Termaat and Munns 1986). Elemental analysis of plant tissue showed that Mn uptake and concentration in the shoot were markedly reduced by salinity and that Mn concentration in the shoot was correlated with relative growth rate (RGR; Cramer et al. 1991). These correlations between Mn and RGR indicate that salinity induced a Mn deficiency, which in turn contributed to the salinity-induced reduction in RGR. In addition, the long-term reduction in relative growth rate (RGR) that was induced by salinity stress was correlated with reduced net assimilation rate (NAR), but not with the leaf area ratio (LAR; Cramer et al. 1990). Thus, the amount of leaf area did not limit growth, but rather an aspect of NAR, such as decreased photosynthesis or increased respiration.

Photosynthesis may be the mechanistic link between Mn deficiency, NAR and RGR since Mn is necessary for the water-splitting enzyme associated with the photosystem II reaction center (Cheniae 1970). Thus, a plausible chain of events is: salinity induces a Mn deficiency, which in turn reduces electron transport capacity, photosynthesis, NAR and finally RGR.

In the present study, we established in a series of three experiments that salinity-induced Mn deficiency can partially limit the growth of salt-stressed barley. First, we verified that supplemental Mn increased growth of salt-stressed barley. Then, we showed that increasing the concentration of Mn in the nutrient solution increased the Mn concentration in the shoot and the RGR of salt-stressed barley. Finally, we showed that this improvement in RGR was associated with an increase in both photosynthesis and NAR.

*Abbreviations* – EC, electrical conductivity; LAR, leaf area ratio; NAR, net assimilation rate; RGR, relative growth rate.

## Materials and methods

### Determination of the effect of supplemental Mn on growth

In the first experiment, barley (*Hordeum vulgare* L. cv. CM 72) was grown in laboratory conditions (25 ± 1°C) under continuous illumination by fluorescent lights [photosynthetic photon flux density (PPFD) = 200 μmol $m^{-2}$ $s^{-1}$]. Seeds were germinated in aerated, distilled-deionized water for 24 h, then placed on plant support systems containing 0.8 $dm^3$ of aerated nutrient solution. The experiment was arranged in a 2 × 7 factorial design with 2 levels of NaCl and 7 levels of Mn. The nutrient solution was either: 1) a one-tenth concentration of modified Hoagland's solution (Epstein 1972) at pH 5.5 (control solution) or 2) a one-tenth modified Hoagland's solution with an additional 125 mol $m^{-3}$ NaCl and 9.6 mol $m^{-3}$ $CaCl_2$ (saline solution). In addition, supplemental Mn treatments were imposed either by increasing the Mn concentration in the nutrient solution or by applying solutions with different Mn concentrations to the foliage. The control Mn concentration was 0.2 mmol $m^{-3}$ in the nutrient solution, and three other Mn concentrations (1.1, 2.0 and 4.0 mmol $m^{-3}$) were obtained by adding $MnSO_4$. For the foliar applications of Mn, plants were grown in nutrient solutions that had 0.2 mmol $m^{-3}$ of Mn. The foliar treatments consisted of 0, 2.5, 5.0 and 10.0 mol $m^{-3}$ of $MnSO_4$ with 0.1% (v/v) of the surfactant Tween 20 (J. T. Baker Co., Phillipsburg, NJ, USA). The foliar treatments were applied to wet the leaves completely at both 7 and 21 days after seeds were planted. All nutrient solutions were replaced with fresh solutions every other day. Plants were harvested 24 days after germination. Each treatment was replicated 4 times in a completely randomized design.

### Determination of the effect of supplemental Mn on Mn uptake, RGR and NAR

In this experiment, barley plants were grown in the greenhouse under a PPFD of approximately 600 μmol $m^{-2}$ $s^{-1}$ (day/night, ca 14/10 h and day/night temperatures of 30/20°C). The experimental design was a 2 × 2 factorial experiment with 2 levels of NaCl and 2 levels of Mn. Plants were germinated as in the first experiment, but were grown for 4 days in control solution. On the 5th day, 12 seedlings were transplanted into a 10-$dm^3$ container that had either control solution or control solution with 2.0 mmol $m^{-3}$ Mn. On day 6, the electrical conductivity (EC) of the nutrient solutions for the salinized plants was raised with a salt concentrate (NaCl/$CaCl_2$ ratio of 13/1) to 7.0 dS $m^{-1}$, which was halfway to the final EC. On day 7, the EC was raised to 14.3 dS $m^{-1}$. Each treatment was replicated 4 times in a completely randomized design. Two plants were harvested from each container on days 8, 11, 14, 17, 20 and 23. Leaf area was measured with a Li-cor (Lincoln, NE, USA) model LI-3000A leaf area meter. Plant material was dried for 48 h at 60°C, and the dry weights of the roots and shoots were measured. Dried shoot material was dry ashed at 600°C for Mn analysis. Shoot Mn was determined with a Perkin-Elmer (Norwalk, CT, USA) model 5000 atomic absorption spectrophotometer. Mn uptake, RGR and NAR were calculated as in previous studies (Cramer et al. 1990, 1991).

### Determination of the effect of supplemental Mn on photosynthesis

In the last experiment, barley plants were grown in a growth chamber set to the following environmental conditions: PPFD = 500 μmol $m^{-2}$ $s^{-1}$ [a mixture of fluorescent (Sylvania cool-white) and incandescent lights (Philips 40 watt)]; relative humidity = 50%; day/night cycle of 12h/12h with temperatures of 25/20°C. The experiment was arranged in a 2 × 2 factorial design with 2 levels of NaCl and 2 levels of Mn. Seeds were germi-

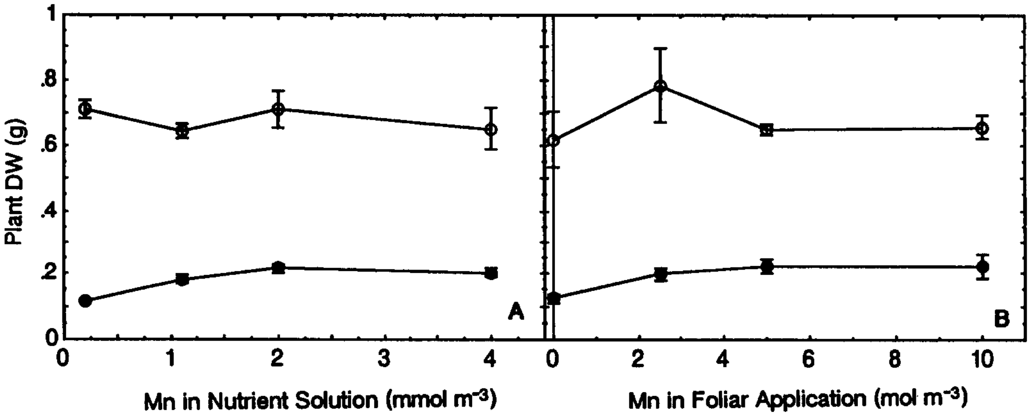

Fig. 1. The effect of supplemental Mn on the growth of salt-stressed barley (experiment 1). A, Supplemental Mn was added to the nutrient solution; B, Supplemental Mn was added as a foliar spplication. ○, control nutrient solution; ●, nutrient solution salinized with 125 mol m$^{-3}$ NaCl plus 9.6 mol m$^{-3}$ $CaCl_2$. In this and following figures, error bars represent the SE; some error bars are smaller than the symbol.

nated as in experiment 1 and planted the next day into 10-dm$^3$ containers with the following 4 treatment solutions: 1) control; 2) control supplemented with 2.0 mmol m$^{-3}$ Mn; 3) salt treatment with an EC of 14 dS m$^{-1}$ (Na/Ca ratio of 13/1) added to treatment 1; and 4) treatment 3 supplemented with 2.0 mmol m$^{-3}$ Mn. Each treatment was replicated 3 times with a completely randomized design. Plants were thinned to 12 plants per container. Two plants were harvested from each container on days 8, 12, 15, 19, 22 and 26. The leaf area and the total dry weight of each plant were measured, and RGR and NAR were calculated as in the second experiment. Photosynthesis measurements were conducted on designated days between harvest dates. Net photosynthetic rates of leaves were measured in a temperature-controlled cuvette and an open $CO_2$ compensation system similar to that of Nowak et al. (1988). All measurements were made in the growth chamber. The temperature of the leaves within the cuvette averaged 24.9°C (SE = 0.1), PPFD averaged 400 µmol m$^{-2}$ s$^{-1}$ (SE = 10), and ambient $CO_2$ concentration within the cuvette averaged 345 µl l$^{-1}$ (SE = 2). A Binos infrared gas analyzer (Inficon Leybold-Heraeus, Hanau, Germany) was used to measure $CO_2$ concentrations. Net photosynthesis was calculated from equations analogous to those in von Caemmerer and Farquhar (1981). The youngest, fully expanded leaf was used for all measurements, and leaf blades from 2 replicate plants were inserted into the cuvette.

## Results

### Effect of supplemental Mn on growth

The objective of this experiment was to determine if increased availability of Mn under saline conditions increased growth. Mn concentration in the nutrient solutions was increased to allow increased Mn uptake. Because Mn transport to the shoot appears to be inhibited by salinity, foliar applications of Mn were also made to insure that Mn concentrations in the shoot could increase.

Supplemental Mn significantly improved the growth of salt-stressed barley plants whether it was applied in the solution or on the leaves (Fig. 1). The optimum Mn concentration for growth under saline conditions was 2.0 mmol m$^{-3}$ in the nutrient solution (Fig. 1A) and 5 mol m$^{-3}$ in the foliar application (Fig. 1B). Although the dry matter production of the salt-stressed plants was doubled by supplemental Mn, supplemental Mn had no significant effect on growth of plants in the control nutrient solutions.

In replicate experiments with $CaSO_4$ substituted for $MnSO_4$, there was no stimulation of growth of plants in either saline or control nutrient solutions (data not shown). Thus, Mn and not S was responsible for the increased growth of salt-stressed plants.

### Effect of supplemental Mn on Mn uptake, RGR and NAR

The objective of this experiment was to provide a more detailed analysis of the effects of supplemental Mn on the growth and Mn nutrition of salt-stressed barley. As in the first experiment, supplemental Mn significantly increased the dry matter production of salt-stressed plants without any significant effect on plants grown in control nutrient solutions (Fig. 2A).

The functional growth analysis approach was used to determine the effects of supplemental Mn on growth. The RGR of salt-stressed plants decreased with time, whereas the RGR of salt-stressed plants with supplemental Mn was maintained (Fig. 2B). Supplemental Mn had no significant effect on the RGR of plants in control solutions. RGR of plants in the control solution was consistently greater than that of plants in the saline nutrient solutions. The increased RGR of salt-stressed plants with supplemental Mn can be attributed to an increase in NAR (Fig. 2C) and not LAR (Fig. 2D). The

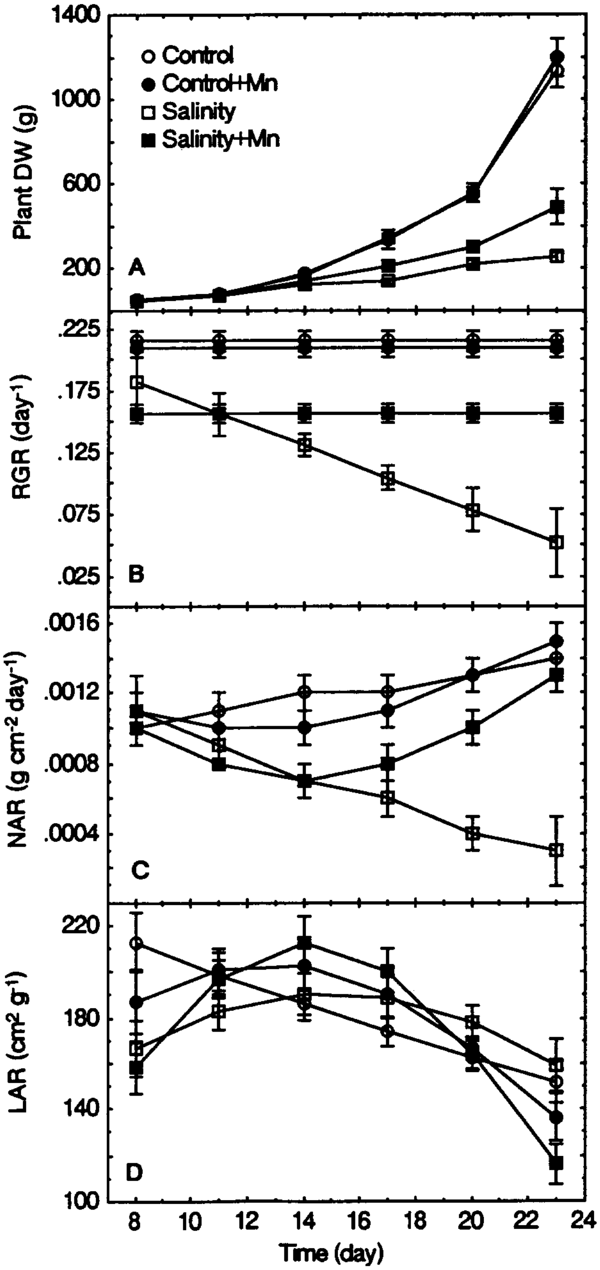

Fig. 2. The effect of supplemental Mn on A, the growth; B, RGR; C, NAR; and D, LAR of salt-stressed barley over time (experiment 2). Symbols are defined in the figure.

NAR of plants grown in control solutions increased slightly with time, but supplemental Mn did not significantly increase NAR for these plants (Fig. 2C). The NAR of salt-stressed plants with supplemental Mn initially decreased to a value below that of the controls, but subsequently increased during the last 9 days of the experiment. In contrast, the NAR of salt-stressed plants without supplemental Mn continually declined with time. By day 17, NAR of salt-stressed plants was significantly below that of salt-stressed plants with supplemental Mn. Except for day 8 and 23, LAR was not significantly affected by the salinity treatments, supporting a previous report (Cramer et al. 1991). The end points of these calculated curves are prone to significant error, and it has been advised that one should interpret these points with caution (Hunt and Parsons 1974).

Supplemental Mn and high salt concentration in the nutrient solution had opposing effects on shoot Mn concentration and uptake (Fig. 3). Supplemental Mn significantly increased the shoot Mn concentration as well as Mn uptake for plants grown in both the control and in the saline nutrient solutions. In contrast, the salinity treatment significantly decreased both the Mn concentration in the shoot and Mn uptake, regardless of the Mn concentration in the nutrient solution. Mn concentrations in the shoot declined with time in all treatments, except for control plants with supplemental Mn (Fig. 3A). In general, Mn uptake tended to increase during the experimental period, except in control plants receiving supplemental Mn (Fig. 3B).

### Effects of supplemental Mn on photosynthesis

The objective of this experiment was to investigate the effects of supplemental Mn on net photosynthetic rate, NAR and RGR of salt-stressed plants. As found in

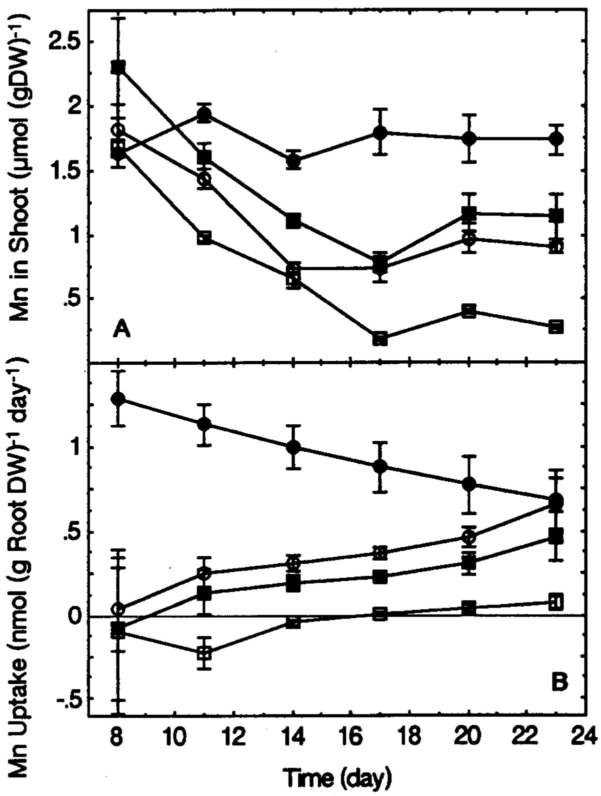

Fig. 3. The effect of supplemental Mn on A, Mn concentrations in the shoot; and B, Mn uptake of salt-stressed barley over time (experiment 2). Symbols are the same as in Fig. 2.

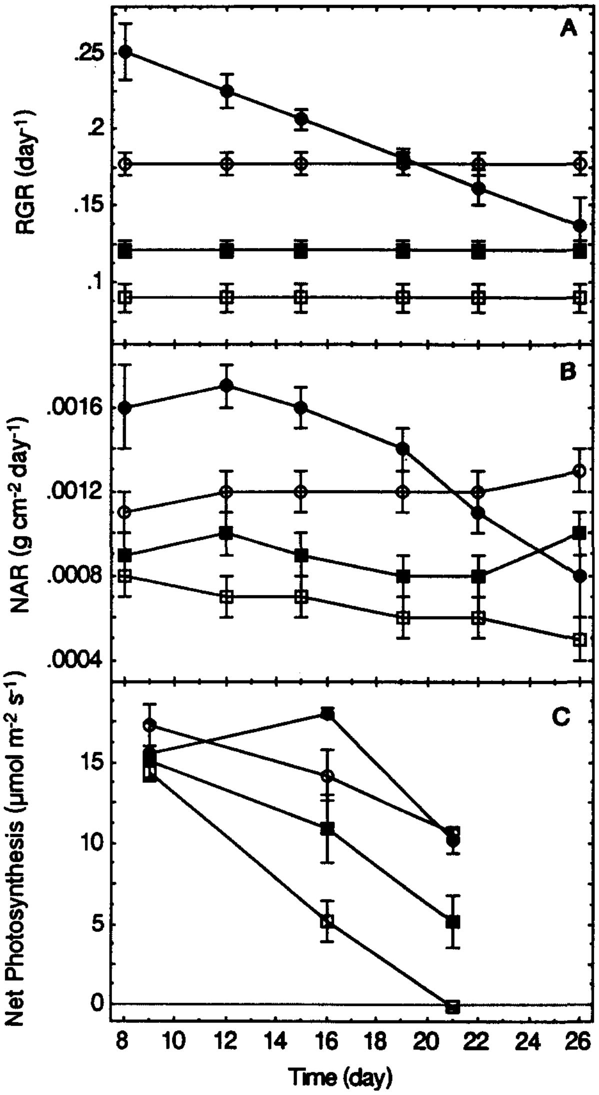

Fig. 4. The effect of supplemental Mn on A, RGR; B, NAR; and C, net photosynthesis of salt-stressed barley over time (experiment 3). Symbols are the same as in Fig. 2.

experiment 2, supplemental Mn significantly increased the RGR (Fig. 4A) and NAR (Fig. 4B) of salt-stressed plants. On close inspection of these results, one can determine minor differences between experiments, which are probably due to differences in experimental condidtions and curve fitting procedures. In most cases, RGR was calculated from a linear fit of the natural logarithmically transformed data, but in two cases a fit to a quadratic equation provided a signifcantly better fit. The quadratic fit results in a declining RGR with time.

Supplemental Mn also increased the net photosynthetic rate of the youngest, fully expanded leaf of salt-stressed plants (Fig. 4C). Net photosynthesis was not significantly different among treatments on the first date that gas exchange was measured (day 9), but ranking of the treatments was identical to that at the end of the experiment. By day 16, differences in both the supplemental Mn and salinity treatments were significant: leaves from plants grown with supplemental Mn had higher net photosynthetic rates than those without, and salinized plants had significantly lower net photosynthetic rates than controls. On day 21, near the end of the experiments, significantly higher net photosynthetic rates with supplemental Mn persisted for plants grown in the saline nutrient solutions, but not for plants grown in the control solutions. Thus, supplemental Mn consistently increased the photosynthetic rate of leaves on salinized plants.

## Discussion

The results of this study support the hypothesis that salinity reduces Mn uptake and concentration in barley shoots, which then causes reduced photosynthetic rates, NAR and RGR. When supplemental Mn is supplied to plants growing in saline nutrient solutions, Mn transport to the shoot, photosynthetic rates, NAR and RGR are all increased. Although supplemental Mn also increased Mn uptake by shoots growing in control solutions, photosynthetic rates, NAR and RGR of these control plants were largely unaffected. Thus, the linkage between Mn nutrition, photosynthesis, and growth is only evident in salt-stressed plants.

The linkage between Mn concentration in the shoot and RGR appears to be through the effects of Mn nutrition on photosynthesis. Three lines of evidence support this hypothesis. First, Mn is necessary for photosynthetic reactions to proceed normally; it is part of the water-splitting enzyme of photosystem II (Cheniae 1970). Thus, the decreased Mn concentration in the shoot may limit enzyme activity. Second, previous studies (Cramer et al. 1990) as well as our current experiments show that decreased RGR is due to decreased NAR, and not decreased LAR. Thus, growth is limited by the ability to fix carbon and allocate that carbon to growth, rather than by the leafiness of the plant. Third, Mn added to saline nutrient solutions increased shoot Mn, photosynthesis, NAR and RGR. Thus, alleviating Mn deficiencies in the shoot improves both photosynthesis and growth. Although the time course of the effect of salinity on photosynthesis differs from that of its effect on NAR, these timing differences can be attributed to the fact that NAR was determined for the whole plant, which includes both older and younger leaves, whereas photosynthesis was determined only for the youngest, fully expanded leaves. In other preliminary experiments, the photosynthetic rate of older leaves was much more affected by salinity than that of younger leaves (data not shown). These observations would explain why NAR was decreased by salinity (Fig. 4B) earlier in the experiment than was photosynthetic rate (Fig. 4C).

Although supplemental Mn improved the growth of salt-stressed barley, Mn uptake and nutrition cannot account for all of the effects of salinity on plant growth. Increasing the concentration of Mn in the nutrient solution to a concentration above 2.0 mmol $m^{-3}$ did not increase growth any further (Fig. 1A). Furthermore, shoot Mn concentrations of plants grown in saline nutrient solutions with supplemental Mn were similar to those of plants grown in the control solution without supplemental Mn (Fig. 3A), but photosynthesis, NAR and RGR differed between these two treatments (Fig. 4). Although the Ca concentration in the saline solution (10 mol $m^{-3}$) was adequate for salt-stressed barley (Cramer et al. 1989, 1990), other ionic and water stresses are also involved in the plant response to salinity. For example, with the removal of both Mn and Ca limitations, salt-stressed barley may be limited by the high cation concentrations in the tissue (Cramer et al. 1990) and some water-stress factor that affects the growth parameters that control growth (Cramer and Bowman 1991).

To our knowledge, the present study is the first to document that salt-stressed plants respond to supplemental Mn. The results support previous findings that Mn defiency could be induced by salinity conditions and consequently limit the growth of barley (Cramer et al. 1990, 1991). The shoot concentration of Mn under control conditions (one-tenth concentration modified Hoagland's solution) was adequate for growth, but inadequate under salinized conditions. However, a full-strength concentration of Mn (2.0 mmol $m^{-3}$), which is still in the low range of most soil solutions (Geering et al. 1969), was sufficient to relieve salinized plants of Mn deficiency. Thus, salinity experiments that use diluted micronutrient solutions may have confounding effects due to salinity-induced micronutrient deficiencies. We recommend that a full-strength concentration of micronutrients be used where plants are to be grown in salinized nutrient solutions.

*Acknowledgements* – We would like to thank Yuqin Lü, Lynn Macaulay and Barbara Seeger for their assistance in some of the experiments conducted in this study.

## References

Cheniae, G. M. 1970. Photosystem II and $O_2$ evolution. – Annu. Rev. Plant Physiol. 21: 467–498.

Cramer, G. R. & Bowman, D. C. 1991. Kinetics of maize leaf elongation. I. Increased yield threshold limits short-term, steady-state elongation rates after exposure to salinity. – J. Exp. Bot. 42: 1417–1426.

– , Epstein, E. & Läuchli, A. 1989. Na-Ca interactions in barley seedlings: relationship to ion transport and growth. – Plant Cell Environ. 12: 551–558.

– , Epstein, E. & Läuchli, A. 1990. Effects of sodium, potassium and calcium on salt-stressed barley. I. Growth analysis. – Physiol. Plant. 80: 83–88.

– , Epstein, E. & Läuchli, A. 1991. Effects of sodium, potassium and calcium on salt-stressed barley. II. Elemental analysis. – Physiol. Plant. 81: 197–202.

Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. p. 39. – John Wiley and Sons, Inc., New York, NY.

Geering, H. R., Hodgson, J. F. & Sdano, C. 1969. Micronutrient cation complexes in soil solution: IV. The chemical state of manganese in soil solution. – Soil. Sci. Soc. Am. Proc. 33: 81–85.

Greenway, H. 1962a. Plant response to saline substrates. I. Growth and ion uptake of several varieties of *Hordeum* during and after sodium chloride treatment. – Aust. J. Biol. Sci. 15: 16–38.

– 1962b. Plant response to saline substrates. II. Chloride, sodium, and potassium uptake and translocation in young plants of *Hordeum vulgare* during and after a short sodium chloride treatment. – Aust. J. Biol. Sci. 15: 39–57.

– 1963. Plant responses to saline substrates. III. Effect of nutrient concentration on the growth and ion uptake of *Hordeum vulgare* during sodium chloride stress. – Aust. J. Biol. Sci. 16: 616–628.

– & Munns, R. 1980. Mechanisms of salt tolerance in nonhalophytes. – Annu. Rev. Plant Physiol. 31: 149–190.

– , Gunn, A., Pitman, M. & Thomas, D. 1965. Plant response to saline substrates. VI. Chloride, sodium, and potassium uptake and distribution within the plant during ontogenesis of *Hordeum vulgare*. – Aust. J. Biol. Sci. 18: 525–540.

Hunt, R. 1982. Plant Growth Curves. An Introduction to the Functional Approach to Plant Growth Analysis. – Edward Arnold, London. pp. 47–120. ISBN 0-7131-2844-5.

– & Parsons, I. T. 1974. A computer program for deriving growth-functions in plant-growth analysis. – J. Appl. Ecol. 11: 297–306.

Munns, R. & Termaat, A. 1986. Whole-plant responses to salinity. – Aust. J. Plant Physiol. 13: 143–160.

– , Greenway, H., Delane, R. & Gibbs, J. 1982. Ion concentration and carbohydrate status of the elongating leaf tissue of *Hordeum vulgare* growing at high external NaCl. II. Cause of the growth reduction. – J. Exp. Bot. 33: 574–583.

Nowak, R. S., Anderson, J. E. & Toft, N. L. 1988. Gas exchange of *Agropyron desertorum*: diurnal patterns and reponses to water vapor gradient and temperature. – Oecologia 77: 289–295.

Termaat, A. & Munns, R. 1986. Use of concentrated macronutrient solutions to separate osmotic from NaCl-specific effects on plant growth. – Aust. J. Plant Physiol. 13: 509–522.

Thiel, G., Lynch, J. & Läuchli, A. 1988. Short-term effects of salinity stress on the turgor and elongation of growing barley leaves. – J. Plant Physiol. 132: 38–44.

von Caemmerer, S. & Farquhar, G. D. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. – Planta 153: 376–387.

Edited by H. E. Sommer