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Effect of pH on Mn absorption by barley genotypes in a chelate-buffered nutrient solution

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Abstract

A new chelate-buffering technique was used to investigate the effect of pH (6.00, 6.85 and 7.70) on manganese (Mn) absorption from nutrient solution by three genotypes of barley plants differing in Mn efficiency. The nutrient composition was adjusted such that the calculated activities of Mn^{2+}, Zn^{2+}, Cu^{2+} and Ni^{2+} were similar in each pH, thus eliminating any effect of the pH treatment on Mn^{2+} supply. Increasing pH from 6.00 to 7.70 increased the rate of Mn absorption and decreased the external Mn requirement for optimal growth rate. With increasing pH, Mn concentrations in roots rose markedly, and were higher than those in shoots at pH 7.70. Genotypic differences in Mn concentration of roots appeared only at higher pH. We suggest that higher Mn concentration in roots of inefficient plants may be related to Mn immobilisation in roots, and this may be a factor in the mechanism of Mn efficiency.

Introduction

Mn deficiency in crops is widespread throughout the world, especially in calcareous soils with high pH (Reuter et al., 1988). In soils, Mn absorption by plants decreases with increasing pH because of a decrease in Mn availability (Marschner, 1988). On the other hand, in nutrient solutions, Mn absorption increased linearly with increasing pH from pH 4.2-6.5 (Munns et al., 1963; Vorm and Diest, 1979), but increasing pH further to the neutral and alkaline ranges, dramatically suppressed Mn uptake because of Mn oxidation and precipitation as suggested by Munns et al. (1963) and Maas et al. (1968). Thus, in order to reliably study the Mn absorption from nutrient solutions of higher pH, it is essential to prevent the precipitation of Mn and other nutrients. The problem of precipitation at higher pH can now be avoided by using a new chelate-buffering technique in conjuction with computer programs such as GEOCHEM (Parker et al., 1993).

Genotypic differences in Mn efficiency have been exploited for high pH soils with low Mn availability (Graham, 1988). However, little is known about the mechanism of Mn efficiency. One possible mechanism of Mn efficiency is genotypic differences in rates of Mn absorption (Graham, 1988). In an earlier experiment, using chelate-buffered nutrient solution at pH 6.0, we found no differences in the specific absorption rate of Mn^{2+} ion among genotypes. However, no reliable information is available on genotypic differences in Mn uptake at higher pH from solution culture.

The following experiment was carried out to study the effect of pH (6.00, 6.85 and 7.70) on Mn absorption by barley plants and to compare three genotypes 'Weeah', 'Schooner', and 'Galleon' differing in Mn efficiency, using a chelate-buffered nutrient solution.

Materials and methods

The computer program GEOCHEM-PC (Parker et al., 1993) was used to calculate nutrient

activities in the solutions. The formation constants of Tris complexes with H^+, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ca^{2+} and Mg^{2+} obtained from Smith and Martell (1989) were included in the GEOCHEM data base. The basal nutrient solution was similar to that described by Webb et al. (1993), but used 5000 μM of different combinations of MES-Tris to buffer 3 levels of pH. Because GEOCHEM-PC predicted that Ca, P and Fe would precipitate at higher pH, the concentrations of $Ca(NO_3)_2$ and $NH_4H_2PO_4$ were reduced to 250 μM and 100 μM, respectively. Similar activity of each micronutrient cation at each pH was achieved by adjusting the total nutrient-chelate supply (Table 1). The activities of Mn, Zn, Cu and Ni expressed as negative log activity (pMetal) were 9.0, 10.5, 14.3 and 15.0. The level of Mn supplied was just below the level predicted as adequate for barley growth in this system at pH 6.00 (Webb et al., 1993). Fe activity was adjusted to pFe 18.8 at pH 6.00 and 6.85, but to avoid precipitation only pFe 19.7 at pH 7.70. To ensure adequate Fe status in all treatments, plants were grown at pFe 17.5 and pH 6.0 until pH treatments were imposed on Day 11 (D11). The maximum pH that could be attained without further compromising nutrient solution composition and still avoiding precipitation, was pH 7.70.

Plant growth and experimental procedures were similar to those described by Webb et al. (1993). On D0, surface sterilized barley seeds were soaked in aerated water overnight and then transferred to seedling cups located in pots containing 900-mL nutrient solution. Plants were grown with 10-hour light/14-hour darkness photoperiod at 15°C day/10°C night. The photon flux density was 500 $\mu mol\ m^{-2}\ s^{-1}$. Each treatment was replicated four times. On D7, seedlings were thinned to 4, 3 and 3 seedlings per cup and solutions renewed. At each harvest (D11, D15 and D19), solutions were changed and plants treated as described in Webb et al. (1993).

Results

At pH 6.00 and pMn 9.0, symptoms of chlorosis appeared on the second leaf in all three genotypes after 17 days. but did not appear at all as pH increased to 6.85 and 7.70. Dry matter production of all three genotypes rose as pH increased (Fig. 1). Mn concentrations in whole shoot tissue increased from 10 to 14 mg kg^{-1} at pH 6.00 to 21 to 27 mg kg^{-1} at pH 6.85 and 7.70 (Fig. 2). With pH increase, the rate of Mn absorption (average of three genotypes±SE) increased from 6.0±1.2 mg root kg^{-1} day^{-1} at pH 6.00, to 17.1±5.0 at pH 6.85 and 22.8±3.8 at pH 7.70 in the period of D15 to D19.

At pH 6.00 and 6.85, Mn concentrations in shoots of Weeah were lower than those of Galleon and Schooner, but not at pH 7.70 (Fig. 2). Manganese concentrations in root tissues increased with increasing pH (Fig. 3). There were no differences in Mn concentrations of

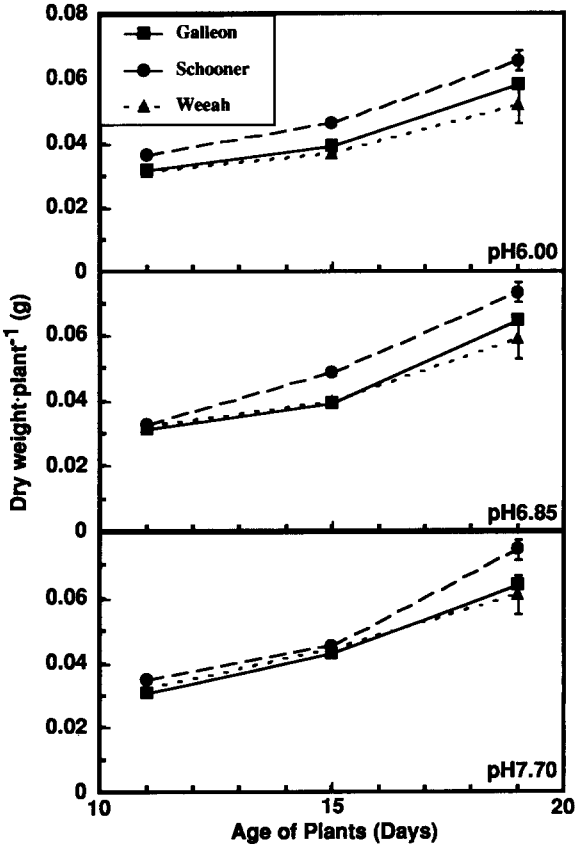

Fig. 1. Effect of three levels of pH on the dry matter production of three barley genotypes grown in a chelate-buffered nutrient solution. SE(n=4) is shown as vertical bars. On D11, plant samples were taken before pH treatments were imposed.

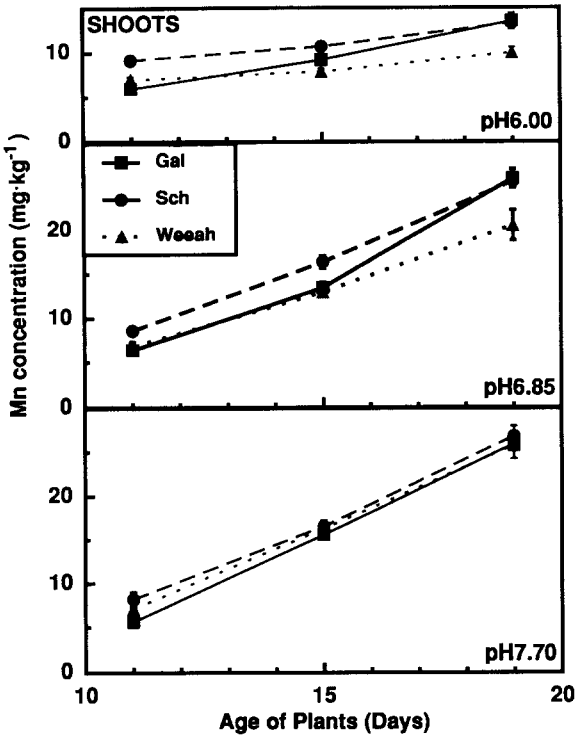

Fig. 2. Effect of three levels of pH on Mn concentrations in shoots of three barley genotypes grown in a chelate-buffered nutrient solution. SE(n=4) is shown as vertical bars. On D11, plant samples were taken before pH treatments were imposed.

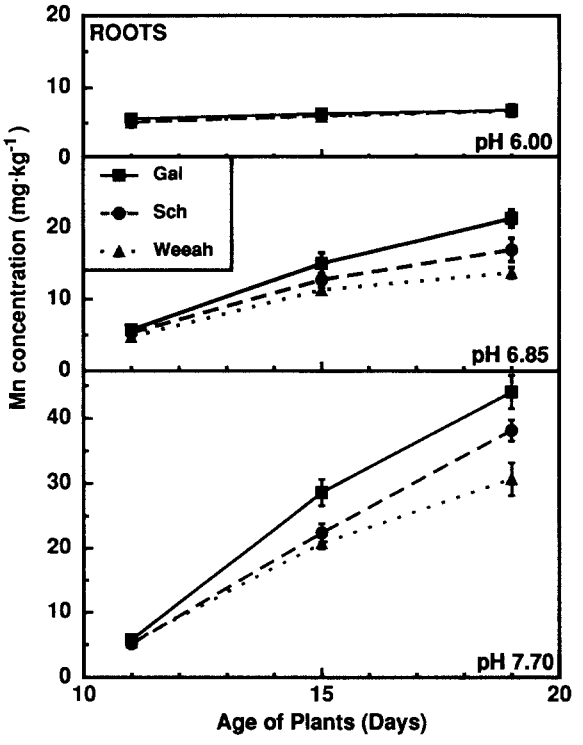

Fig. 3. Effect of three levels of pH on Mn concentrations in roots of three barley genotypes grown in a chelate-buffered nutrient solution. SE(n=4) is shown as vertical bars. On D11, plant samples were taken before pH treatments were imposed.

roots among genotypes at pH 6.00, but there were at both 6.85 and 7.70. Mn concentrations in roots were higher than those in shoots (Fig. 2) after 8 days treatment in pH 7.70.

Discussion

The rate of Mn absorption from the nutrient solutions was greatly increased by pH, in the current chelate-buffered nutrient solution. However, in 10 μ*M* Mn of a single salt solution, Mn absorption reached a maximum at pH 7.0, and remained constant in the range of pH 7.0 to 8.7 (Ratkovic and Vucinic, 1990), although Mn activity did not change with pH increase. This may be related to the saturation of the absorption pathway at high pH because of the high concentration of Mn used. In conventional nutrient solutions, however, a depression in the rate of Mn absorption from 5 μ*M* occurred when pH rose above 6.0 (Munns et al., 1963). This depression has been attributed to Mn oxidation and precipitation (Munns et al., 1963; Maas et al., 1968), or may be related to co-precipitation or adsorption with other nutrients such as Fe (Bell et al., 1991). In the chelate-buffered nutrient solution that we used, micronutrient cations were complexed with the ligand, and concentrations of free ions were very low, constant and similar in each pH. Fe was not likely to have precipitated from solutions because high concentrations of Fe in the roots was not found (data not shown). Thus, the complicating factors which may influence Mn absorption were eliminated in this experiment. Therefore, the increase in Mn absorption with increasing pH was probably related to a lack of H^+ competition and/or to an increasing H^+ efflux gradient in this experiment.

Table 1. Compositions of pretreatment and pH treatments.

Treatment	Nutrient solution composition (μM)				
	Fe	Mn	Zn	Cu	Ni
Day 1-Day 11					
pH 6.00	50.0	0.215	10.0	1.0	0.1
Day 11-Day 19					
pH 6.00	2.5	0.215	10.0	1.00	0.1
pH 6.85	20.0	0.250	11.5	1.15	0.1
pH 7.70	20.0	0.260	12.0	1.20	0.1

An increase in pH apparently decreased the minimum external Mn requirement of barley. Barley needed pMn 8.3 at pH 6.0 (Webb et al., 1993), but pMn 9.0 was adequate for barley plants at pH 6.85 in this experiment. This lowering of the external requirement may help plants survive in high pH soils with low Mn availability.

The low concentration of Mn in roots at pH 6.00 is likely to be a consequence of the high concentration of competing chelate which would scavenge any free Mn^{2+} in the root apoplasm. Thus, the higher Mn concentration in roots with increasing pH, in both absolute terms and relative to shoot concentrations, suggests that Mn was somehow immobilised in roots. However, it is not clear which cellular component may be involved in such Mn immobilisation: MnO_2 precipitation in apoplasm, or some immobilised form of Mn in cytoplasm or vacuoles.

Genotypic differences in Mn concentration of roots appeared at higher pH. That Mn concentration in roots of Weeah was lower than that of Galleon could, therefore , suggest that either less Mn is immobilised or more Mn is remobilised. In either case, it would be expected that more Mn is available to shoots of Weeah. The efficient cultivar, Weeah, appears to be performing relatively better at high pH than low pH when compared to the inefficient Galleon. This may help explain the difference in Mn efficiency in the field which is most outstanding in high pH soils.

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