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Mobilisation of iron and manganese by plant-borne and synthetic metal chelators

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Abstract

Phytosiderophores released by roots of iron-deficient grasses mobilise Fe, Zn, Mn and Cu in calcareous soils. Mobilisation of Fe, Zn and Cu can be explained as the chelation of these metal cations by phytosiderophores. Mobilisation of Mn could not be so explained because phytosiderophores have a much smaller affinity for Mn than for Fe, Cu and Zn. Model experiments have been made with freshly precipitated $Fe(OH)_3$ and different soils to study the mobilisation of iron and manganese by plant-borne chelating phytosiderophores, the synthetic metal chelators DTPA and the microbial metal chelator sulphonated ferrioxamine B (FOB). Compared with the synthetic chelator DTPA, the plant-borne chelating phytosiderophores mobilised Fe very efficiently, but no change was observed in the Mn mobilisation by phytosiderophores.

Different phytosiderophores, as well as the microbial metal chelator FOB, were used to compare the mobilisation of iron and manganese in a calcareous soil.

Introduction

Phytosiderophores (PS) are released by cereal roots in response to low Fe and Zn availability (Takagi, 1976; Zhang et al., 1989). Further, whilst Fe mobilisation from freshly precipitated $Fe(OH)_3$, or from a calcareous soil, could be demonstrated (Awad et al., 1988, Zhang et al., 1991), mobilisation of Zn, Cu, and Mn could also be shown (Treeby et al., 1989). Thus PS released by Fe- or Zn-deficient grass increase the rhizosphere concentrations of chelated micronutrient cations.

The present investigation was undertaken to study in more detail, the efficiency of Fe mobilisation by PS, and the mobilisation of Fe and Mn by different PS (mugineic acid (MA); 2'-deoxymugineic acid (DMA); epi-3-hydroxymugineic acid (HMA); avenic acid (AA), synthetic chelator (DTPA) and a microbial siderophore, sulphonated ferrioxamine B (FOB).

Materials and methods

Seeds of barley (*Hordeum vulgare* L.cv. Europa and Minorimugi), wheat (*Triticum aestium* L. cv. Ares) and oats (*Avena sativa* L. cv. Pirol) were germinated in $CaSO_4$-saturated quartz sand for three days in the dark, prior to being transferred to 2.5 L plastic vessels (60 plants per vessel) with continuously-aerated Fe-free nutrient solution (pH 6.2; 2.00 m*M* Ca $(NO_3)_2$; 0.75 m*M* K_2SO_4; 0.65 m*M* $MgSO_4$; 0.25 m*M* KH_2PO_4; 0.10 m*M* KCl; 1 μ*M* H_3BO_3; 0.5 μ*M* $MnSO_4$; 1 μ*M* $ZnSO_4$; 0.25 μ*M* $CuSO_4$; 0.005 μ*M* $(NH_4)_6 Mo_7O_{24}$)]. The plants were grown under controlled environmental conditions [day/night, 16/8 h; light intensity, 220 mol m^{-2} s^{-1} (Fluorescent tubes Sylvania, cool white FR 96T 12); temperature, 25°/23°C; relative humidity, 70-80%] and the nutrient solution was changed every second day.

Root exudates were collected after the onset of

Fe-deficiency symptoms in the foliage (at day 7). At the beginning of the light period in the morning, the plant roots were removed from the nutrient solution, rinsed briefly in distilled water and placed into aerated, distilled water for 4 h. The plants were then returned to the nutrient solution. The washing solution containing the root exudates was filtered using blueband filters, concentrated under vacuum at 40°C to 20 mL, and stored at -18°C.

Root exudates were dissolved in a small volume of deionised water, passed through an exchange resin (Amberlite IR 120 (H^+)) and the fraction eluted with 1*M* NH_4OH was collected. The fractionation of the different PS and the identification by HPLC followed the method of Kawei et al. (1987).

The microbial chelator FOB was obtained from Ciba-Geigy GmbH, Germany. Analytical grade DTPA was obtained locally.

The PS content was quantified by measuring the amount of Fe(III) dissolved from $Fe(OH)_3$ as described by Takagi (1976). Briefly, an aliquot of isolated PS was shaken with freshly-precipitated $Fe(OH)_3$ for 2 h at 25°C, soluble Fe was measured photometrically as Fe(II) Ferrozine at 562 nm following the addition of a reductant (hydroxylamine hydrochloride) and Ferrozine [3-(2-pyridyl)-5,6-bis (4-phenylsulfoine acid)-1,2,4-triazinel].

The soils used in the experiments were 2 calcareous and 2 acidic soils from China and a Luvisol from Germany. The soil properties and DTPA-extractable micronutrient cation contents, determined according to Lindsay and Norvell (1978), are given in each of the figures.

Mobilisation of micronutrient cations was assayed as follows: a 20 mL aliquot of PS or chelators was shaken with 2 g of air dried soil for 3 h at 25°C and centrifuged. The supernatant was passed through a 0.1 µm millipore filter and the content of micronutrient cations measured by atomic absorption spectrophotometry. For all treatments there were 5 replicates, and mean values are presented in the Figures. Mobilisation was measured as Fe equivalents.

Results

PS (DMA) was highly efficient in mobilising Fe from sparingly soluble $Fe(OH)_3$, compared with the synthetic chelator DTPA (Fig. 1). The mobilisation of Fe by DMA was complete within 30 minutes, but the mobilisation of Fe by DTPA required more than 72 h. Similar patterns of Fe mobilisation by DMA and DTPA were also demonstrated in a calcareous soil (Fig. 2). When chelators were added to soil, much more Fe in solution was found in the presence of DMA than with DTPA at the initial phase. The maximum mobilisation of Fe of DMA was reached after 5 h, after which, the mobilisation of Fe declined.

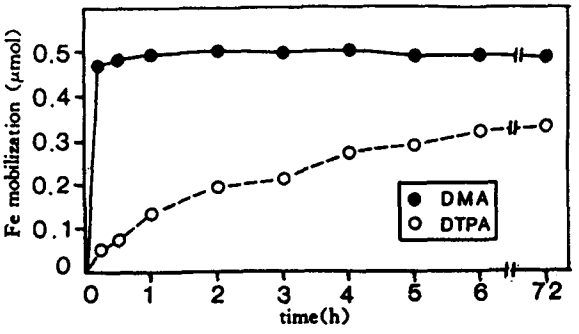

Fig. 1. Time course of Fe mobilisation by DMA and DTPA (0.5 µmol) from freshly precipitated $Fe(OH)_3$.

The mobilisation rate of Fe by DTPA increased rapidly immediately after the addition of the chelators and continued to increase with time for the whole experimental period.

When the concentration of metal chelator was

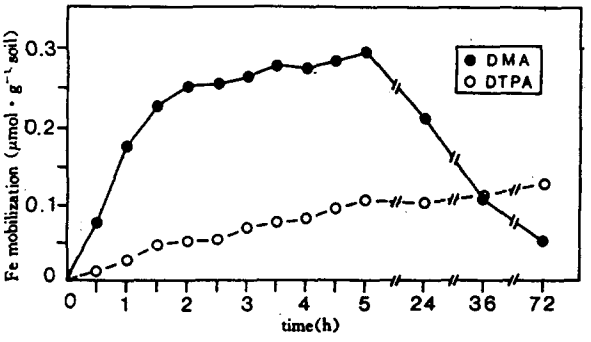

Fig. 2. Time course of Fe mobilisation by DMA and DTPA (4.4 µmol) from a calcareous soil (pH: 8.0; DTPA-Fe: 4.1 mg/kg).

lower than 1μmol, DMA mobilised much more Fe than DTPA (Fig. 3). As the concentration of chelators increased, the mobilisation of Fe in the same soil by both chelators also increased, but the increase in the rate for DMA was much smaller than that for DTPA.

The effect of soil pH on the mobilisation of Fe from a calcareous soil by DMA, FOB and DTPA were compared (Fig. 4). Of the three chelators used, DMA gave the highest mobilisation of Fe. This mobilisation capacity was affected to a lesser extent when the soil pH was adjusted from 6 to 8. Fe mobilisation by FOB was lower than

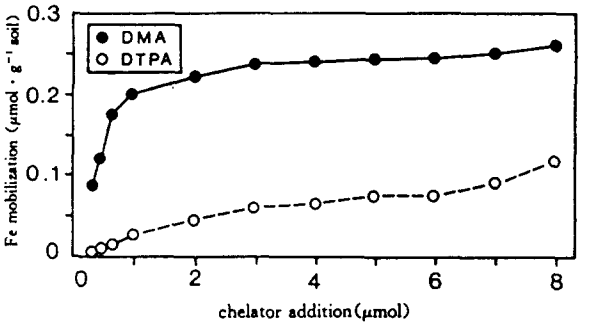

Fig. 3. Effect of DMA and DTPA additions on the Fe mobilisation from a calcareous soil (see Fig. 2).

by DMA and declined slightly when pH was raised. The greatest depression of Fe mobilisation with increased pH was found in the DTPA treatment. For example, as the soil pH increased from 6 to 8, the mobilisation of Fe by DTPA decreased more than 3 times.

Another experiment showed that the sterilisation of the soil had a strong increasing effect on the mobilisation of Fe (Fig. 5).

Besides the microbial activity of a soil, other soil properties influence the mobilisation rate of Fe. The effect of soil properties on the Fe and Mn mobilisation with DMA and DTPA were compared using 2 different soils (Fig. 6). The highest Fe mobilisation observed in these 2 soils were by DMA, but the Mn mobilisation by DMA varied with different soils. No Mn mobilisation by DMA was found in soil A; even in soil B, containing a very high amount of exchangeable Mn, DMA mobilised a very small amount of Mn compared with DTPA.

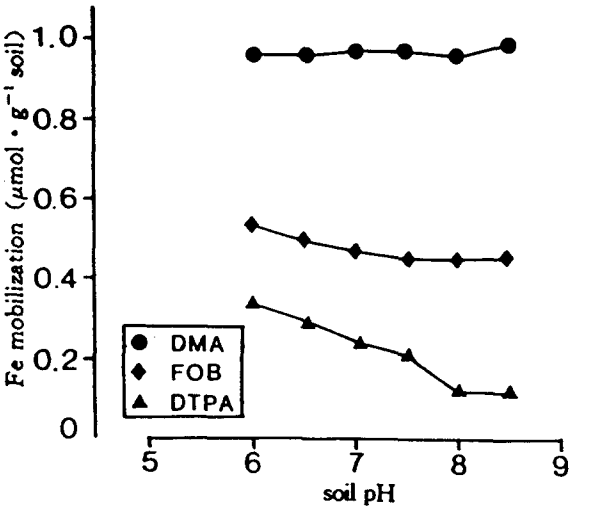

Fig. 4. Effect of soil pH on the mobilisation of Fe by DMA, FOB and DTPA from a soil (pH:6.0; DTPA-Fe:8.0 mg/kg). Soil pH was adjusted by addition of lime.

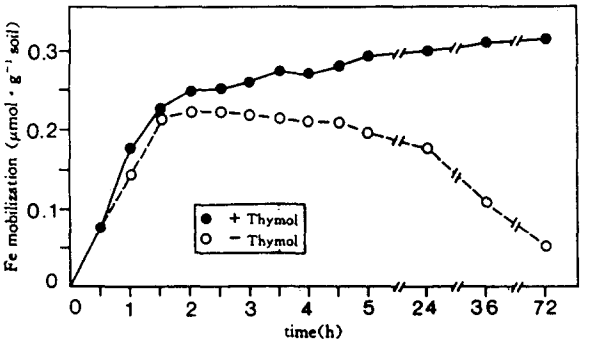

Fig. 5. Effect of thymol on the mobilisation of Fe from soil (see Fig. 2) by DMA (4.4 μmol DMA, 15 mg thymol).

In another experiment with Luvisol (Fig. 7), the mobilisation of Fe by all phytosiderophores (DMA, HMA, MA and AA) was much higher than that by DTPA and FOB, whereas the mobilisation of Mn was much higher with DTPA than that by other chelators.

Discussion

The efficiency of Fe mobilisation by PS from soils can be affected by several factors. These include: binding of PS to metal ions other than Fe; inhibitory effect of high pH and calcium

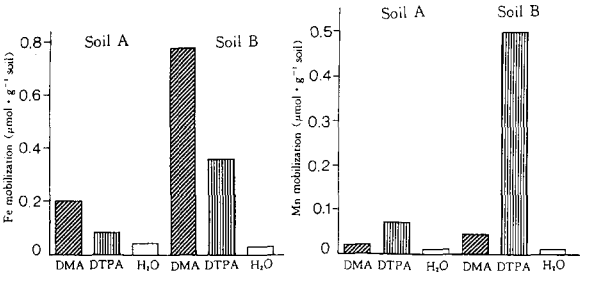

Fig. 6. Fe and Mn mobilisation by DMA and DTPA from a calcareous soil (soil A: pH:8.1, DTPA-Fe:6.3 mg/kg; DTPA-Mn:3.1 mg/kg) and a acidic soil (soil B: pH:5.50,DTPA-Fe:11.8 mg/kg, DTPA-Mn:40.9 mg/kg, Exchangeable Mn:6500 mg/kg).

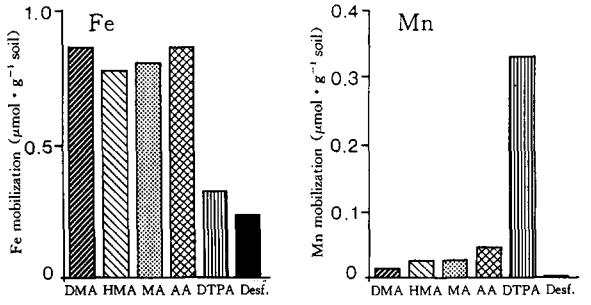

Fig. 7. Fe and Mn mobilisation by different phytosiderophores (2.5 μmol) from a Luvisol (pH, 6.0; $CaCO_3$, 7.7%; organic matter, 2.0%; DTPA-Fe 16.2 mg/kg; Mn, 30.7 mg/kg).

content in calcareous soils; degradation of PS by microorganisms in the rhizosphere; and adsorption of PS to soil particles. Only parts of these factors are discussed here. Compared with the synthetic chelator DTPA, the plant-borne chelator PS mobilised Fe very effectively. The efficient mobilisation of Fe was characterised by fast mobilisation from freshly precipitated $Fe(OH)_3$, which was completed within 30 minutes (Fig. 1). This efficient mobilisation of Fe by PS could also be observed in a calcareous soil (Figs. 2, 3, 4). This may be assigned not only to the fast mobilisation of Fe by PS (Fig. 2) and the much higher Fe-extracting efficiency of PS compared with the synthetic chelator (DTPA) (Fig. 2), but also the smaller effect of increased soil pH on the Fe mobilisation by PS (Fig. 4).

Previous research has shown that although other micronutrients (Cu, Zn, Mn) are also mobilised in the soil by PS (Takagi et al., 1988; Treeby et al., 1989), application of these micronutrients, even as inorganic salts to calcareous soils, has only a slight inhibitory effect (Cu) or even no effect (Zn, Mn) on Fe mobilisation by PS (Zhang et al., 1991). Römheld (1991) estimated that on average, mobilised Fe accounts for 20-40% of the total micronutrients mobilised by PS.

Degradation of PS by microorganism has been confirmed in the current experiment (Figs. 4, 5). Römheld (1991) has argued that, in addition to a distinct diurnal rhythm in PS release (Takagi et al., 1984; Marschner et al., 1986), the spatial separation of PS release and microbial activity in the rhizosphere should partly compensate for microbial degradation.

In agreement with the results from Takagi et al. (1988), no Mn mobilisation was found in the calcareous or acidic soils of our experiment (Fig. 7). We conclude that, under certain experimental conditions, PS mobilise Fe by chelation very efficiently, but not Mn.

Acknowledgments

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