# An ESR Study of Manganese Binding in Plant Tissue

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**Abstract.** Two different fractions of manganese were found in the maize plant root apoplasm (intercellular space containing cell walls) after soaking the roots in $MnCl_2$ solutions (concentration range 0 01–10 mmol.$l^{-1}$): (*a*) an $Mn^{2+}$ fraction in the water free space (WFS) which gave a characteristic six-line spectrum, and (*b*) an immobilized fraction that gave no detectable ESR spectrum. Both fractions affect proton NMR relaxation ($T_1$) of the tissue water through water exchange across cell membranes. ESR spectra of free and total manganese of the root tissue treated with $MnCl_2$ also revealed different time courses for saturation of WFS and DFS with $Mn^{2+}$. Binding of manganese in the extracellular space of the tissue seems to be the rate limiting step in permeation of $Mn^{2+}$ across the root cell membranes

**Key words:** ESR — $Mn^{2+}$ — Plant tissue — Ion binding — DFS — WFS

## Introduction

The selective absorption of ions in Donnan free space (DFS) of a root apoplast is a controlling step in the ion uptake and transport through a plant (Haynes 1980; Lütge and Higinbotham 1979). The affinity of cations for negatively charged exchange sites in DFS is proportional to charge of cations, and it was shown that passive physico-chemical adsorption is of importance for the uptake of divalent cations.

There have been few investigations of $Mn^{2+}$ transport through plant roots (Page and Dainty 1964; Maas et al. 1968; Ramani and Kannan 1975; Bowen 1981). In these studies manganese content of plant material was mostly analyzed using radioactive $^{54}Mn$ or by atomic absorption spectroscopy (Bačić and Ratković

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1984). On the other hand, methods like electron spin resonance (ESR) and nuclear magnetic resonance (NMR) were employed in some studies of the state of manganese in plant leaves and chloroplasts (Wydrzynski et al. 1978; Van As et al. 1980).

In our previous NMR studies of $Mn^{2+}$ transport across the primary root of *Zea mays L.* (Bačić and Ratković 1984; Ratković and Bačić 1987) it was assumed that the first phase of ion uptake is diffusion of ions within the apoplast-intercellular space containing cell walls. In this paper we present an ESR study of $Mn^{2+}$ binding in roots of *Zea mays* in initial phases of absorption. The ESR results were correlated with some NMR and AAS data.

## Materials and Methods

*ESR measurements*

The ESR spectra were recorded on a Varian E-9 ESR spectrometer under following conditions: microwave frequency 9.3 GHz, microwave power 20 mW, modulation frequency 100 kHz, modulation amplitude 10 G, time constant 3 s.

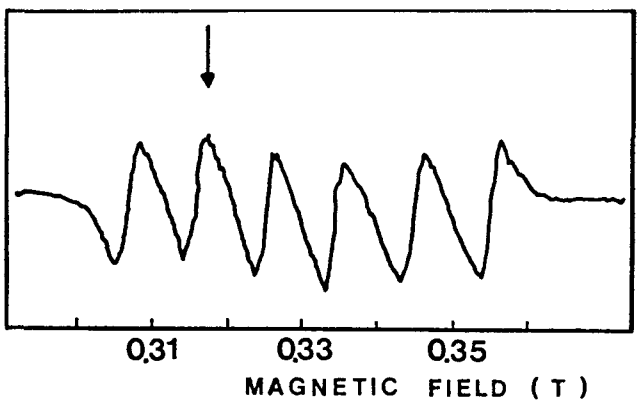


**Figure 1.** The ESR spectrum of $Mn^{2+}$ in a root of *Zea mays L.* soaked in 0.1 mmol.$l^{-1}$ $MnCl_2$ for 60 min. The arrow indicates peak 2; its amplitude was taken as a measure of signal intensity.

The paramagnetic properties of $Mn^{2+}$ ion are due to its electron structure ($3d^5$) with unpaired electrons ($S = 5/2$), and this enabled easy detection of ESR spectra (Dwek 1975). The characteristic six-lines spectrum of free aquo-ion of $Mn^{2+}$ in root tissue is shown in Fig. 1. Bound manganese would be expected to give a broad ESR line with a superimposed spectrum of free $Mn^{2+}$. Meanwhile, bound manganese could not be detected directly due to intensive line broadening. The relative amounts of manganese

in roots were calculated from the second peak amplitude (Fig. 1) normalized on the root weight and instrumental amplification.

Two types of measurements were performed: (*a*) the roots were preloaded with manganese by soaking in 0.01–10 mmol.l$^{-1}$ $MnCl_2$ solutions over different time intervals; subsequently they were cut into small pieces, placed in ESR sample tube and the ESR spectra were recorded. (*b*) The same pieces of the roots were digested in a mixture of mineral acids ($HNO_3$/HCl), the solution was then evaporated to a volume close to that of the original root sample and transferred to ESR sample tube.

*NMR measurements*

Proton spin-lattice relaxation time, $T_1$, of root tissue water was measured on a pulsed NMR relaxometer (modified model IJS-2-71) at resonance frequency 32 MHz and with a standard pulse sequence $180° - \tau - 90°$ (Farrar and Becker 1971).

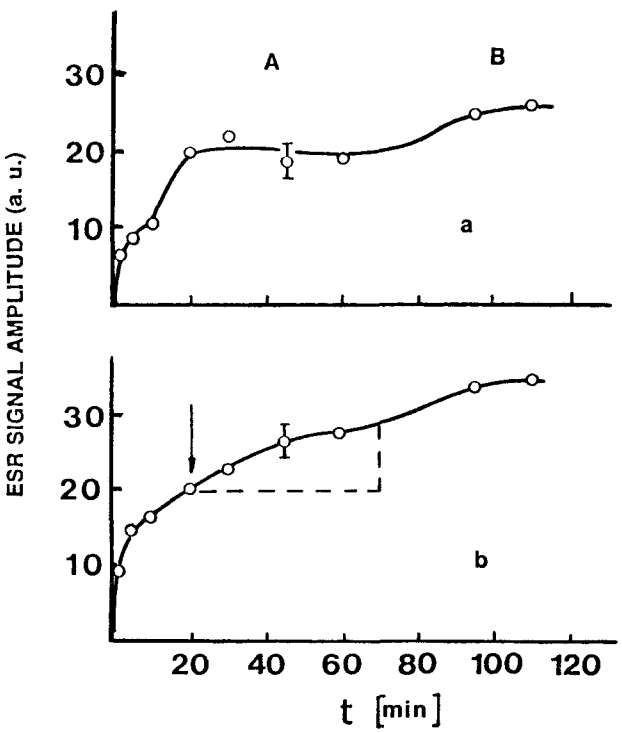


**Figure 2.** Changes in relative ESR signal amplitude in dependence on the soaking time in 1 mmol.l$^{-1}$ $MnCl_2$ solution: (*a*) intact roots - ESR signal for free $Mn^{2+}$, (*b*) digested roots - ESR signal corresponding to total manganese (free+bound).

*Analysis of manganese by AAS*

The manganese contents of plant material were estimated by atomic absorption spectrophotometry on a Pye Unicam SP-192 spectrometer after digesting the samples in a mixture of mineral acids and dilution in HCl. The analyses were done in acetylene-air flame at 279.5 nm wavelength.

*Plant material*

The experiments were done with primary roots of the maize hybrid ZPSC 46A (low-salt roots). The seeds were germinated 4 days in Petri dishes between layers of wet filter papers at 25 °C, and the bottom 5 cm from the tip were taken for analysis.

## Results and Discussion

Fig. 2*a* shows changes in ESR signal intensity for $Mn^{2+}$ in intact maize roots soaked in 1 mmol.l$^{-1}$ $MnCl_2$ solution for almost 2 h. Two phases can be distinguished on the uptake curve (A and B) similarly to our previous NMR and AAS studies on $Mn^{2+}$ uptake by maize roots (Bačić and Ratković 1984): saturation of the root apoplast, i.e. of the intercellular space including cell walls, with $Mn^{2+}$ ions probably corresponds to the first plateau *A*, and was terminated within 20 min (Fig. 2*a*). Times much longer than 20 min would be required for complete saturation of the

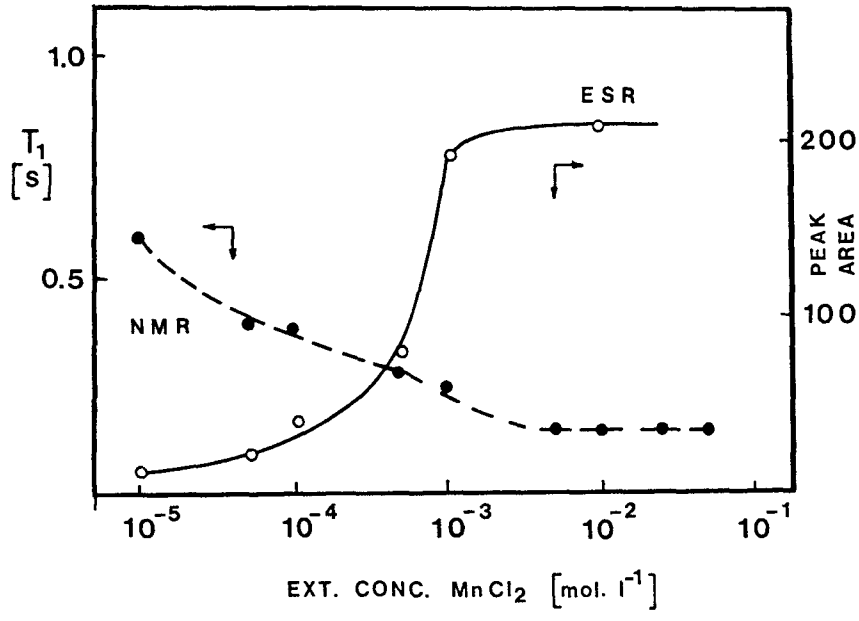


**Figure 3.** The concentration dependence of the ESR peak intensity and proton NMR $T_1$ of tissue water for roots of *Zea mays L.* soaked in $MnCl_2$ solutions (0.01–50 mmol.l$^{-1}$).

root tissue with manganese; this is shown in Fig. 2*b* with total manganese having been transferred to the ESR-visible ionic form by digestion of the manganese-loaded root segments. Direct comparison of the ESR amplitudes in Figs. 2*a* and 2*b* is not quite justified because of the possible differences in geometry between the intact root samples and digested samples. Nevertheless, we can compare the two curves shown in Fig. 2 in a kinetic sense evaluating the half-time of saturation of phase $A, t_{1/2} = 10$ min.

When the area under the second ESR peak (Fig. 1) was plotted against external $MnCl_2$ concentration (0.01–10 mmol.l$^{-1}$) a curve was obtained as shown in Fig. 3. The shape of the curve, particularly at lower concentrations, suggests binding of Mn within the root tissue. While ESR signal intensity increases with the increasing $MnCl_2$ concentration, proton $T_1$ of tissue water measured by NMR decreases, due to the presence of $Mn^{2+}$ ions as relaxation centers for water protons (Bačić and Ratković 1984). There is a plateau above 1 mmol.l$^{-1}$ $MnCl_2$ in both the ESR and the NMR curve. The proton NMR data agree with our previous results on relaxation in root tissue loaded with different concentrations of $MnCl_2$ and $Mn(EDTA)^{2-}$ (Bačić and Ratković 1984).

Also distribution of $Mn^{2+}$ between water free space (WFS) and Donnan free space (DFS) of the root apoplast has been calculated for external $MnCl_2$ concentrations in the 0.01–1.0 mmol.l$^{-1}$ range (Ratković and Bačić 1985). Based on these

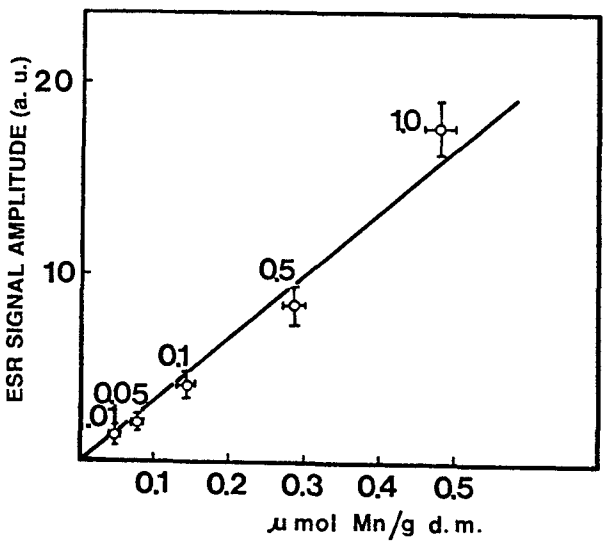


**Figure 4.** The ESR signal intensity of $Mn^{2+}$ in maize roots corresponding to phase *A* in Fig. 2, plotted against $Mn^{2+}$ concentration in WFS. The figures at the line refer to the concentration of the external $MnCl_2$ solution.

results, the ESR signal amplitude was plotted against $Mn^{2+}$ concentration ($\mu$mol Mn/g dry matter) in WFS for different external concentrations of $MnCl_2$ (Fig. 4). The linear dependence is still another indication in support of only unbound $Mn^{2+}$ giving detectable ESR signal. The ESR results presented in this paper, together with the NMR results on the same tissue, support the view that binding of divalent cations within the cell walls and within the intercellular space (Haynes 1980) is an important step in the process of ion uptake by plant roots.

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