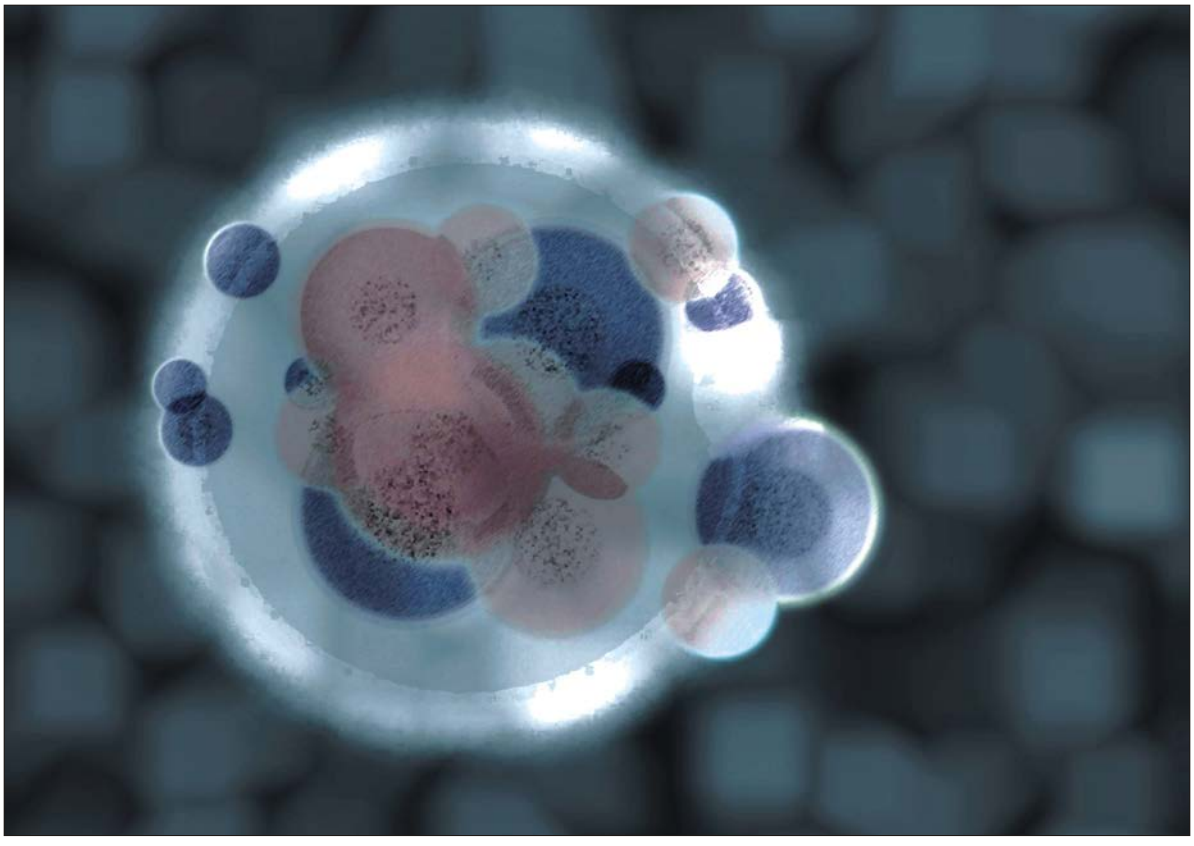

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Kinetics of Zinc Uptake in Sorghum (*Sorghum bicolor* L., Moench) Roots Infected with Arbuscular Mycorrhizal Fungus, *Glomus macrocarpum*

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Abstract: The kinetics of Zn uptake was studied in arbuscular mycorrhizal (*Glomus macrocarpum*) and non-mycorrhizal roots of sorghum (*Sorghum bicolor* L. Moench). Within Zn concentration range, 10 μmol to 100 mmol m^{-3}, five concentration dependent phases of Zn absorption were identified. Phase 0 (0.05-0.1 mmol m^{-3}) was linear but the other four subsequent phases (0.1 mmol to 0.1 mol m^{-3}) conformed to Michaelis-Menten kinetics. At extremely lower concentrations (below 0.05 mmol m^{-3}), Zn absorption followed a linear pattern. Mycorrhizal roots invariably maintained higher Zn absorption rate than non-mycorrhizal root. Higher Zn absorption rate of mycorrhizal roots appeared to be related to higher maximal uptake rate (V_{max}) for phase 1, 3 and 4 and greater specificity (lower km) for phase 2 (1 to 5 mmol m^{-3}).

Key words: Multiphasic uptake, Zn uptake isotherm, *Sorghum bicolor* L., *Glomus macrocarpum*

INTRODUCTION

Arbuscular Mycorrhizal (AM) plants are capable of absorbing higher amounts of micronutrients such as Zn and Cu than their non-mycorrhizal counterparts even under suboptimal supply of these nutrients in soil (Ryan and Angus, 2002; Sharma and Johri, 2002; Kothari *et al*., 1991). Higher absorption of nutrients by mycorrhizal plants is generally attributed to much wider exploration of the soil volume by extramatricular hyphae (Rhodes and Gerdemann, 1975). Improved uptake of P, Zn and Cu in mycorrhizal corn has been ascribed to hyphal uptake (Kothari *et al*., 1991). Mycorrhizal roots have also been shown to differ in nutrient uptake characteristics (Bowen *et al*., 1974; Cress *et al*., 1979). Sharma *et al*. (1992) have demonstrated that arbuscular mycorrhizal corn roots show a multiphasic uptake kinetics of Zn which maintained higher uptake due to higher maximal uptake rate (V_{max}) in low concentration range but greater specificity (lower k_m) in subsequent higher Zn concentration. In the present investigation, we have examined concentration-dependent uptake kinetics of Zn for mycorrhizal and non-mycorrhizal sorghum roots.

MATERIALS AND METHODS

Source of arbuscular mycorrhizal fungus: The pure inoculum of *Glomus macrocarpum* maintained in autoclaved soil-sand mixture (2:1) on maize roots for six months in glass house which used for the experimental purpose.

Glass house experiment: Hydrochloric acid-washed quartz sand (2 kg) was sterilized by autoclaving for 2 h at 121°C and employed to fill eight plastic pots (dia. 15 cm). Half of the pots were inoculated with AMF by placing 5 g inoculum containing 100 spores plus infected root segments and hyphae just below each seed site. The spores were washed and treated with chloramine T and streptomycin (Schenck, 1982) before inoculating the plants. Remainder pots (control) were supplied with 50 mL spore wash solution. In each pot, ten sorghum seeds germinated on towel paper were sown. All pots were kept in a glasshouse with day and night temperature maxima of 35 and 25°C, respectively. All pots were supplied with a modified Hoagland solution containing 0.025 g Zn m^{-3} (half of the normal dose). The pots were irrigated with double distilled water. All plants were harvested at 45 day after sowing.

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Mycorrhizal and non-mycorrhizal roots were carefully removed from ten pots, washed with deionized water and placed in 2 mol m^{-3} $CaCl_2$ solution containing 1/10 strength modified Hoagland solution. Both mycorrhizal and non-mycorrhizal roots were segmented (0.5-2.5 cm) behind root tip with the help of a razor. Both mycorrhizal and non-mycorrhizal segments were kept separately. Fifty randomly selected roots segments were stained with toludine blue O and acid fuchsin (Sharma *et al*., 1988) and examined under a microscope (x100) for % mycorrhizal infection (Biermann and Lindermann, 1981).

Zinc uptake experiment: Twenty root segments, mycorrhizal or non-mycorrhizal, were weighed and separately kept inside a nylon bag and tied with nylon thread. The enclosed root segments were placed in 50 cm^3 of 0.5 mol m^{-3} $CaCl_2$ solution containing 10 μ mol to 100 mmol Zn m^{-3} tagged with ^{65}Zn (specific activity 925 MBq g^{-1}) in duplicate for 1 h at 25°C. The solutions were stirred intermittently. After the uptake period, the enclosed roots segments were dipped in 50 cm^3 of 0.5 mol m^{-3} $CaCl_2$ solution for 5 min followed by desorption in an identical but unlabelled solution for 5 min. The root segments were gently pressed between blotting papers, dried at 70°C for 24 h, weighed and counted for ^{65} Zn activity on solid scintillation counter.

RESULTS AND DISCUSSION

Mycorrhizal sorghum roots scored 75.7% for infection while non mycorrhizal roots were free from any fungal association. Zinc uptake by sorghum roots was resolved into five distinct concentration dependent phases (Fig. 1). In connection range (0.05-0.1 mmol m^{-3}), Zn uptake increased linearly (phase 0) however, below 0.05 mmol Zn m^{-3} the uptake was better represented stripped line with a different slope. Phase 1 was noted in the concentration range 0.1 mmol to 0.75 mmol m^{-3} but it also showed a definite saturation. The transition between phase 0 and phase 1 was not distinct as the former phase merged smoothly into the later. Phase 2 was noticed in the range 1-5 mmol m^{-3} and the transition between phase 1 and phase 2 occurred between 0.75 mmol and 1 mmol m^{-3}. Phase 3 and 4 were noticed in Zn concentration range of 7.5-25 mmol m^{-3} and 25-100 mmol m^{-3}, respectively. The transition between phase 2 and 3 was noted between 5 mmol and 7.5 mmol m^{-3} while the transition between phase 3 and 4 was not distinct.

Invariably, mycorrhizal roots absorbed Zn at much faster rate than non-mycorrhizal roots. The increase in Zn uptake due to mycorrhizal association was relatively higher in the concentration range 0.5 mmol to 1.0 mmol m^{-3}. Sharma *et al*. (1992) have earlier demonstrated higher effectiveness of AMF inoculated maize to absorb Zn in low concentration range.

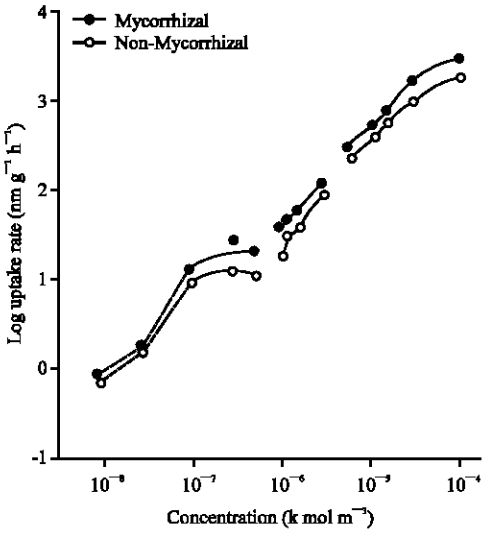

Fig. 1: Uptake isotherms of Zn by sorghum (*Sorghum bicolor*) root (fresh weight basis) at 1×10^{-8} to 1×10^{-4} k mol Zn m^{-3} stripped lines depict lower end of phase 0. Phase 1-4 were fitted to Michaelis-Menten equation (thick lines)

Table 1: Kinetic constants for different phases of Zn uptake by mycorrhizal (AM^+) and non-mycorrhizal (AM^-) roots of sorghum (*Sorghum bicolor* L. Moench)

	Michelis constant (Km mol m^{-3})		Maximal uptake rate (V_{max} μmol g^{-1} fresh wt. g^{-1})	
Phase	AM^+	AM^-	AM^+	AM^-
1	7.59×10^{-5}*	2.76×10^{-5}	0.023*	0.012
2	0.001*	0.003	0.092	0.083
3	0.146**	0.039	5.589**	1.438
4	0.222	0.191	8.333**	4.693

*: $p<0.005$, **: $p<0.01$ mycorrhizal versus non-mycorrhizal

The straight line relationships in Lineweaver-Burk plots (Fig. 2), phase 1 to 4 conformed to Michaelis-Menten kinetics. Kinetics constants; maximal uptake rate (V_{max}) and Michelis constant (k_m), for different phases shown in Table 1 show that mycorrhizal roots had significantly higher V_{max} and k_m values than non-mycorrhizal roots for phase 1 and 3.

In phase 2, V_{max} for mycorrhizal roots did not differ significantly from those for non-mycorrhizal root but k_m value was certainly lower for mycorrhizal root. Further in phase 4, V_{max} value for mycorrhizal root was higher than non-mycorrhizal roots but km values did not differ significantly.

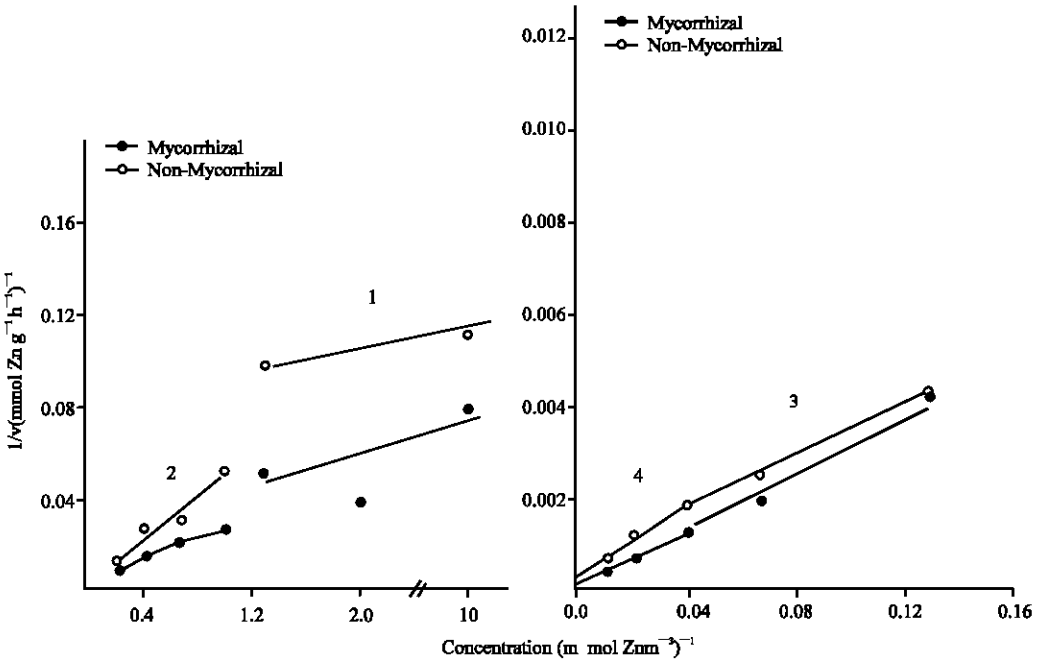

Fig. 2: Lineweaver-Burk plots for Zn uptake data (v) of phases I to IV in Zn concentration (S) range of 0.1 to 10.0 mmol m^{-3}

Zinc uptake time adopted in this investigation was 1 h and the desorption period was only 10 min. Nissen (1973) noted that the quasi-steady overall flux might serve as useful estimate of influx across the plasmalemma at external concentrations below 10 mol m^{-3}. Kinetic patterns of solute uptake for a short period of time have been found similar to those found in long period experiments (Nandi *et al*., 1987).

In this study, sorghum roots irrespective of arbuscular mycorrhizal association exhibited five concentration dependent Zn uptake phases within the range of 0.01-100 mmol m^{-3}. In an earlier study, Sharma *et al*. (1992) noted five phases for Zn uptake by corn roots in the concentration range of 0.075-1070 mmol m^{-3}. Zinc concentration ranges reported for different uptake phases by corn (*Zea mays* L.) roots differed from those reported in this investigation for sorghum. The variation could be ascribed to differences in plant species, the age of the plant and procedure followed.

Mycorrhizal association in sorghum proved to be more effective in increasing Zn uptake only in low concentration range, especially between 0.1-1.0 mmol m^{-3}. In general, higher Zn uptake rate of mycorrhizal sorghum roots was related to the larger number of carrier sites. However, in a limited concentration range of 1.0-5 mmol m^{-3}, greater specificity (k_m) of the carrier seemed to be more important in maintaining increased uptake rates. Sharma *et al*. (1992) noted that Zn uptake by mycorrhizal and non-mycorrhizal roots was related to large number of carrier sites in the concentration range of 4-10 mmol Zn m^{-3} and beyond this concentration, greater specificity of carrier(s) played more important role in increasing Zn uptake rates. The apparent differences in the findings of present investigation and those of Sharma *et al*. (1991) could be attributed to the factors mentioned in the preceding paragraph.

Thus, it could be accomplished from the present findings that myccorhizal roots invariably maintained higher Zn absorption rate than non mycorrhizal roots. However, the kinetics of Zn uptake by mycorrhizal and non-mycorrhizal roots was found to be multiphasic in the sorghum. It could be due to the plant species which seems to exercise definite control on uptake pattern of Zn rather than arbuscular mycorrhizal fungus.

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