coded by *pat* gene and *npt*-II gene, respectively. Our investigation has also shown that the *GUS* gene-expressing cells were mainly located in the subepidermal layer between the epidermis and the vascular bundles. Similar reports are available for *Pisum*[20]. There seems to be a tissue-specific competence to *Agrobacterium* infection in the subepidermal cell layers. With regard to our observations, we suggest that the subepidermal cells undergo rapid, wound induced dedifferentiation and cell division, which leads to a high competence for infection by *Agrobacterium* and a very high transformation rate.

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Multiphasic zinc uptake system in mycorrhizal and nonmycorrhizal roots of French bean (*Phaseolus vulgaris* L.)

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Kinetics of Zn uptake by vesicular arbuscular mycorrhizal (*Glomus macrocarpum*) and nonmycorrhizal roots of French bean (*Phaseolus vulgaris* L.) were investigated employing ^{65}Zn. Within 10 μmol to 100 mmol Zn m^{-3} solute concentration, five concentration-dependent Zn uptake phases were resolved. Zinc uptake by roots was linear in the range 0.053–0.1 mmol Zn m^{-3} (phase 0). However, at extremely low concentration (below 0.05 mmol m^{-3}), Zn uptake followed a sigmoidal pattern. In the concentration range, 0.1 mmol to 100 mmol m^{-3}, four other distinct uptake phases each one following Michaelis–Menten kinetics were observed. Mycorrhizal roots exhibited generally higher Zn absorption rate than the nonmycorrhizal roots; this is attributed to higher maximal uptake rates (V_{max}) for phases 1, 2 and 3. The greater specificity of phase 4 was a result of a lower k_m value.

ZINC is an essential element for several enzyme systems that regulate various metabolic activities in plants. It is involved in auxin production which is vital for the growth process in plants, and helps in transformation of carbohydrates and regulates sugar in plants. As a consequence of zinc deficiency, as much as 50% reduction in crop growth can result without the appearance of visual symptoms and therefore, it is considered as the most important micronutrient in crop production. Application of zinc increased ascorbic acid in potato tubers, reduced phenol content and enhanced reducing sugars, sucrose and total sugars in potato[1]. Increased energy value, total lipids, crude protein and carbohydrate content in rice, maize, wheat, *raya*, groundnut, chickpea and blackgram were noticed with zinc application[2]. The association of vesicular arbuscular mycorrhizal (VAM) fungi with higher plants has been found to result in higher Zn absorption than their nonmycorrhizal counterparts[3–5]. This is ascribed to much wider exploration of soil volume by extramatricular hyphae of mycorrhizae and improved uptake of nutrients by hyphae[4,12]. Higher Zn uptake rate in mycorrhizal roots has been demonstrated in corn[6,7]. This could be explained on the basis of higher V_{max} (maximal uptake rate) in low concentration range and

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lower k_m (greater specificity) in the subsequently higher Zn concentration range. The present investigation examines the concentration dependent uptake kinetics of Zn by mycorrhizal and nonmycorrhizal French bean (*Phaseolus vulgaris* L.) roots with a view to assessing if the multiphasic kinetics are also followed in the legume system.

Pure culture of *Glomus macrocarpum*, (provided by K. V. B. R. Tilak, IARI, New Delhi), was maintained in autoclaved soil–sand mixture (2:1) on maize roots for 6 months in a glasshouse.

Hydrochloric acid-washed quartz sand (2 kg) was sterilized by autoclaving for 2 h at 121°C and employed to fill eight plastic pots (15 cm diameter). Seeds of French bean were germinated in a paper towel; ten seeds were placed in each pot. VAM spores were surface sterilized with chloramine T and streptomycin[8]; 200 spores were placed in a hole below each seed site. Control pots were supplied with 50 ml spore wash solution. All pots were kept in a glasshouse with day and night temperature maxima of 35 and 25°C, respectively. Pots were supplied with a modified Hoagland solution weekly with half the normal recommended dose until the end of the experiment. Irrigation was carried out with double distilled water. All plants were harvested 45 days after sowing.

Mycorrhizal and nonmycorrhizal plants were gently removed to avoid any injury to the roots and tapped to dislodge any adhering sand. They were placed separately in water-filled plastic buckets. A continuous water stream was maintained in the bucket to ensure complete removal of adhering sand from the roots. This procedure was specifically adopted to collect the intact root system without any mechanical damage. Roots were carefully washed with deionized water and placed in 2 mol m^{-3} $CaCl_2$ solution containing 1/10 strength modified Hoagland solution. Both mycorrhizal and nonmycorrhizal roots were cut, 0.5–2.5 cm behind the root tip with a sharp razor. Fifty randomly selected root segments were stained with toluidine blue O and acid fuchsin[9] and examined under a microscope (× 100) for mycorrhizal infection[10].

Twenty root segments (each segment was examined for the presence of extramatricular mycelium), both mycorrhizal and nonmycorrhizal, were weighed, 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 µmol to 100 mmol Zn m^{-3} tagged with ^{65}Zn (specific activity 925 MBq g^{-1}) for 1 h at 25°C; treatments were carried out in duplicate and solutions were stirred intermittently. After the uptake period, root 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 Zn solution twice each for 5 min and finally in distilled water 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 a solid scintillation counter.

French bean roots were infected by VAM to an extent of 76.9% whereas nonmycorrhizal segments were free of any fungal infection. Zinc uptake in French bean roots appeared to follow five distinct concentration-dependent phases (Figure 1). In the concentration range 0.05–0.1 mmol Zn m^{-3}, uptake rate increased almost linearly (phase 0), however, below 0.05 mmol Zn m^{-3} the uptake pattern followed a nearly sigmoidal pattern (stripped line in Figure 1). The uptake phase 1 was observed in 0.1 mmol to 0.75 mmol m^{-3} of Zn with a definite indication towards saturation. The transition between phase 0 and phase 1 was not clearcut; the former phase merged smoothly into the later phase. Phase 2 was operative in the range 1.0 mmol to 5.0 mmol Zn m^{-3} and the transition between phase 1 and 2 occurred between 0.75 mmol and 1.0 mmol Zn^{-3}. Subsequent phases (phase 3 and 4) were noted in the Zn concentration range, 7.5 mmol–25 mmol m^{-3} and 25 mmol to 100 mmol m^{-3}, respectively. The transition between phase 2 and phase 3 occurred between 5 mmol and 7.5 mmol m^{-3}; transition between phase 3 and phase 4 was, however, not distinct.

Mycorrhizal roots absorbed Zn at a much faster rate than nonmycorrhizal roots at all concentrations. A double reciprocal plot of Zn uptake rate versus Zn concentration (Lineweaver–Burk plot) for phases 1 to 4 showed a straight line as described by Michaelis–Menton type kinetics (Figure 2). The values of maximal Zn uptake rate (V_{max}) and Michaelis constant (k_m) for different uptake phases are given in Table 1. A comparison of V_{max} and k_m values revealed that higher Zn uptake rate of mycorrhizal roots were a result of significantly higher V_{max} values for phases 1, 2 and 3 and a lower k_m value for phase 4.

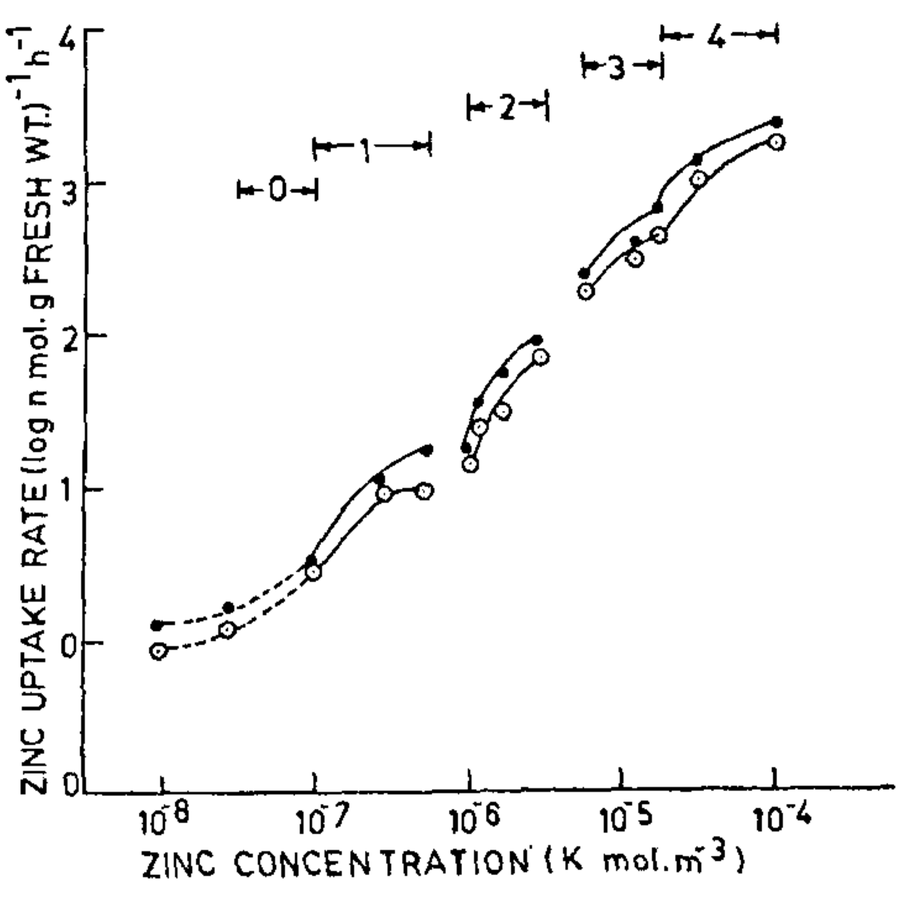

Figure 1. Zinc uptake isotherms of French bean (*Phaseolus vulgaris* L.) at 1×10^{-8} to 1×10^{-4} K mol Zn m^{-3}. Stripped lines indicate lower end of phase 0. Phase 1 to 4 were filled to Michaelis–Menten equation (thick lines). • mycorrhizal roots, o non-mycorrhizal roots.

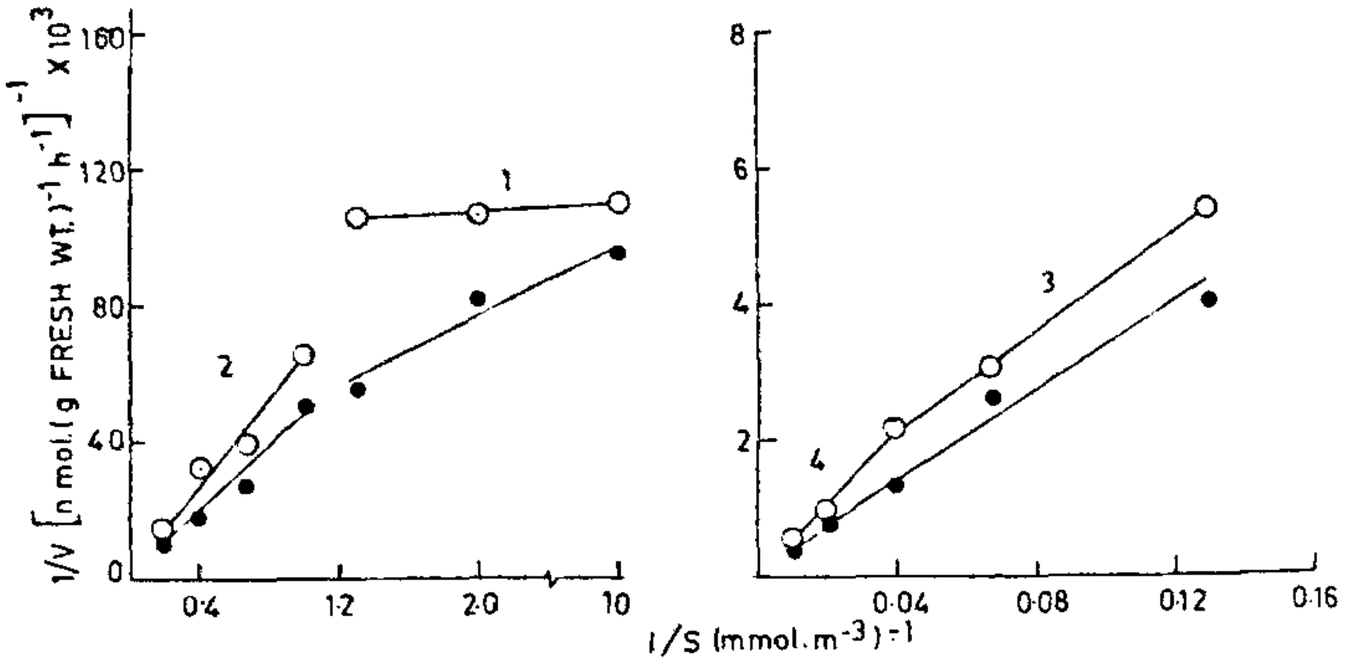

Figure 2. Lineweaver–Burk plots for Zn uptake data (V) of phase 1 to 4 in Zn concentration (s) range of 0.1 to 100 mmol m^{-3}. • mycorrhizal roots, o non-mycorrhizal roots.

Table 1. Kinetic constants for different phases of Zn uptake by mycorrhizal (VAM^+) and nonmycorrhizal (VAM^-) roots of French bean (*Phaseolus vulgaris* L.)

	Michaelis constant (k_m, mol m^{-3})		Maximal uptake rate (V_{max}, µmol g^{-1} fresh wt)	
Phase	VAM^+	VAM^-	VAM^+	VAM^-
1	5.54×10^{-5}**	0.515×10^{-5}	0.016*	0.009
2	0.012*	0.005	0.314**	0.091
3	0.077*	0.048	2.687*	1.343
4	0.174*	0.437	6.056	9.413

*$P < 0.05$, **$P < 0.01$ mycorrhizal versus nonmycorrhizal.

Zinc uptake period adopted in the present investigation was 1 h and desorption period, 10 min. At low solute concentration (below 10 mol m^{-3}), the quasi-steady overall flux has been reported to be an estimate of ion influx across the plasmalemma[11]. Five distinct concentration-dependent phases were observed for both mycorrhizal and nonmycorrhizal roots within a range of 0.01–100 mmol Zn m^{-3}. In an earlier work on Zn uptake kinetics of mycorrhizal and nonmycorrhizal corn roots, Sharma *et al.*[7] have described the presence of five uptake phases of Zn in the concentration range of 0.075–1070 mmol Zn m^{-3}. Therefore, a legume system does not appear different from non-legume system at least with respect to short term uptake kinetics of Zn.

Higher Zn uptake rate of mycorrhizal roots in the concentration range 0.1 to 25 mmol Zn m^{-3}, can be ascribed to the large number of carriers. At 25 to 100 mmol Zn m^{-3}; greater specificity (lower k_m) of mycorrhizal roots appeared important in the resultant higher uptake rate. For corn roots also, Sharma *et al.*[7] noted that higher specificity was more important for mycorrhizal roots to sustain higher Zn uptake rates at 10 mmol Zn m^{-3}. As compared to corn, French bean exhibited much greater uptake rate and a lower specificity. Available data suggest that when multiphasic uptake kinetics are operative in mycorrhizal roots, the nature of plant species will influence the maximal uptake rates and specificity of the carrier. Larger number of carrier sites appear to be responsible for improved Zn uptake of mycorrhizal French bean at lower Zn levels.

Thus, kinetics of Zn uptake by mycorrhizal and nonmycorrhizal French bean roots were found to be multiphasic. The nature of plant species has a profound effect on maximal uptake rate and specificity of the carrier. Higher number of carrier sites appears to be the main reason for higher Zn uptake rate of mycorrhizal French bean roots at lower Zn concentrations.

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