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Kinetics of zinc uptake by two rice cultivars

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Summary Rice (*Oryzae sativa* L.) cultivars differ widely in their susceptibility to zinc (Zn) deficiency. Excised root apices of cv IR26 actively absorbed Zn at a rate twice that of cv M101 roots. This difference in Zn uptake rates could not be attributed to greater root surface area in cv IR26 as compared to cv M101. The maximum rates of Zn uptake (Vmax) and the Km values also differed markedly between these two cultivars. Roots of cv M101 have a two-fold greater affinity for Zn than do those of cv IR26. Leaf blade tissues of IR26 and M101 rice absorbed Zn at similar rates. Rice cv IR26 readily develops Zn deficiency symptoms in hydroponic culture but cv M101 rarely does so.

Introduction

Nitrogen, phosphorus and zinc (Zn) deficiencies are the most widespread and economically most important nutritional factors limiting growth and production of wetland rice[8,12]. Zinc deficiency occurs commonly in rice-producing areas on sodic, calcareous and organic soils, and soils that are frequently wet for prolonged periods of time[8,12]. It is thus of great practical importance to rice breeders to identify cultivars that are Zn-efficient; *i.e.*, that are capable of thriving on soils that have available Zn concentrations that would usually be considered inadequate.

Scientists at the International Rice Research Institute in the Philippines have been screening rice cultivars for several years in an effort to select those that are Zn-efficient[8,9,12]. Several such cultivars have been found and some studies have been done on the physiological mechanism that enables these to grow on soils with less than optimal Zn levels[5,6,11,13].

The Zn nutrition of other crops, such as sugarcane[1,2,16] and barley[14], has been studied to a greater extent, however. The purpose of the present study was to extend the previous work with rice and to provide further insight into the physiology of Zn efficiency in this culturally and economically important crop.

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Materials and methods

Plant material

The two rice (*Oryzae sativa* L.) cultivars used in these experiments were IR26, thought to have a high Zn requirement, and M101, considered to require much less Zn for optimum growth (G S Khush, personal communication). Cultivar IR26 is a tropical rice of the Indica ecogeographic race whereas cv M101 is a temperate-zone Japonica (Sinica) rice. These two cultivars would not normally be compared in yield experiments but were selected for study herein solely on the basis of their relative Zn requirements.

Apical 1 cm sections of roots from 10-day-old seedlings were prepared as described previously for barley roots[3]. The maximum time lapse between excision and use was 1 h.

Leaf sections (300 u × 14 mm) were cut from the center third of elongating leaf blades of 8-week-old rice plants grown in vermiculite in the greenhouse in the same way as was done earlier with sugarcane[1].

The root apices and leaf blade sections (approximately 1 g fr wt of each), in cheesecloth 'teabags', were immediately placed in constantly-aerated 0.5 m*M* $CaSO_4$ solution for a maximum of 1 h before the start of an experiment. (Rice does not require or benefit from aeration of culture solutions[8,9,12], nor did aeration affect Zn uptake in these experiments (data not presented). Solutions were routinely aerated only so that these data would be directly comparable to those from other Zn uptake studies.)

Experimental methods

Root apices and leaf blade sections were placed for up to 2 h in continuously-aerated solutions containing 0.5 m*M* $CaSO_4$, non-radioactive Zn as $ZnSO_4 . 7H_2O$ (0.10 m*M* to 0.50 m*M*) and ^{65}Zn (0.75 uc ^{65}Zn/umole non-radioactive Zn; sp. act. of ^{65}Zn was 178 uc/umole). The initial pH of each solution was 5.7 ± 0.2, and the temperature was 26° ± 1°.

At the end of the uptake period, the plant tissue was rinsed three times for 1 min each in flowing distilled water, and then desorbed for 30 min in aerated 0.5 m*M* $CaSO_4$ at 4°[1,2,14]. Thus, the reversibly-adsorbed fraction was eliminated and the Zn uptake rates reported herein pertain only to metabolically mediated active absorption[1,2,14].

The plant tissue was then weighed, dried overnight at 75°, and ashed at 400° for 12 h. The ash was redissolved in 0.1 *N* HCl, and a 1 ml aliquot was assayed for radioactivity with a scintillation probe equipped with a NaI (TI) crystal (3 cm diameter × 2.5 cm thick) connected to a scaler.

Each experiment contained three replicates and was run a minimum of four times.

Root surface area was estimated with the method of Dittmer[4] and also by direct measurement from photographs of excised root apices.

Kinetic analyses

The kinetic constants Km and Vmax were determined by the double reciprocal plot method[10] and were reproducible to within ± 15 percent.

Statistical analyses

Student's 't-test' was used for testing the statistical significance of differences in Km and Vmax between treatments and cultivars[15]. All curves in Figures 1 to 5 were fitted statistically by computer, using the Tell-a-Graf program[7].

Results

Zn uptake by rice roots as a function of time

Figure 1 depicts the active uptake[1,2] of Zn by excised root apices of two rice cultivars as a function of time. The external Zn concentration in these experiments was 0.1 m*M*. Active Zn uptake by root apices from

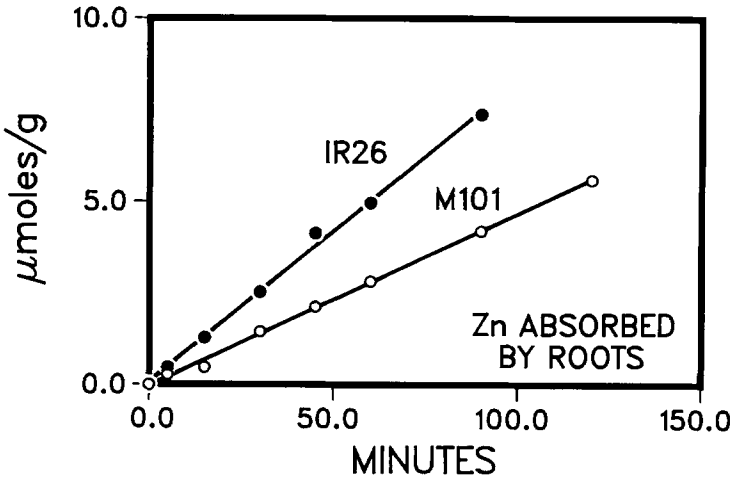

Fig. 1. Active absorption of zinc by excised root apices from rice cultivars IR26 and M101 as a function of time. External solution contained 0.10 m*M* Zn as $ZnSO_4 . 7H_2O$ and 0.50 m*M* $CaSO_4$; pH 5.7 ± 0.2; temperature 26° ± 1°; desorption 30 min in 0.50 m*M* $CaSO_4$ at 4°.

both Zn-efficient rice (cv M101) and Zn-inefficient rice (cv IR26) was linear with time for at least 2 h (Fig. 1). The rate of Zn absorption by cv IR26 roots was approximately twice as rapid as that by M101 roots, however (Fig. 1). Similarly results were obtained when the external Zn concentration was 0.50 m*M* (data not shown).

Subsequent experiments were run with a 30 min absorption period, well within the linear response range, unless otherwise noted.

Zn uptake by rice roots vs external Zn concentration

Uptake rates for Zn by root apices from cvs M101 and IR26 were measured as a function of the external Zn concentration over the range of 0.01 m*M* to 0.50 m*M* Zn (Fig. 2). The tissue Zn levels increased sharply when exogenous Zn was increased from 0.01 m*M* to about 0.10 m*M* (Fig. 2). Root apices from the two cultivars did differ significantly (1 percent confidence level) in their capacity to absorb Zn over this concentration range although, in each case, a curve typical of other micro-nutrient cationic uptake systems was observed (Fig. 2)[1,2,14].

Roots of cv IR26 consistently absorbed Zn at a rate approximately twice that of cv M101 roots (Fig. 2). The Zn uptake system in each rice cultivar was saturated at an exogenous Zn concentration of 0.10 m*M* to 0.20 m*M* (Fig. 2). This observation is in accord with earlier reports on Zn uptake in sugarcane[1,2].

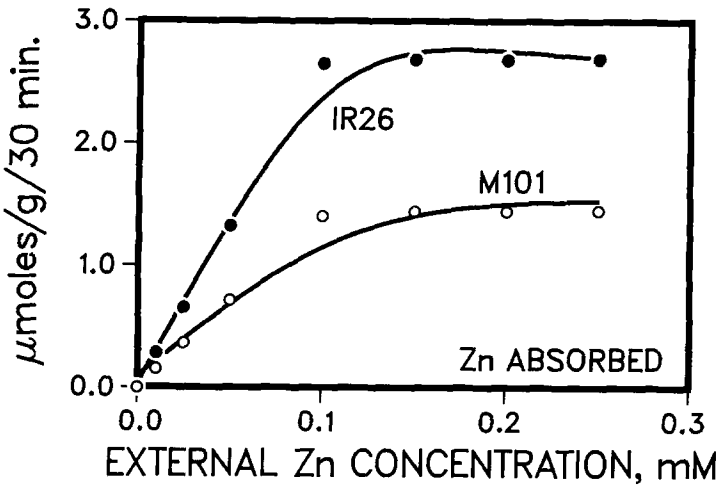

Fig. 2. Uptake of Zn by excised root apices from rice cultivars IR26 and M101 as a function of the external Zn concentration. $CaSO_4$ concentration, 0.50 m*M*; absorption period, 30 min; pH 5.7 ± 0.2; temperature, 26° ± 1°. Desorption, 30 min in 0.50 m*M* $CaSO_4$ at 4°.

The initial Zn concentration in the root apices of cv M101 was 0.70 umoles/g dry wt and that in cv IR26 roots was 0.56 umoles/g dry wt. The effect of this difference upon subsequent Zn absorption is unknown.

Kinetics of Zn uptake by rice root apices

The kinetic parameters Vmax (umoles Zn absorbed/g fr wt/30 min) and Km (m*M*) were determined from double reciprocal plots of the data presented in Fig. 2 (Table 1). The maximal rate of Zn uptake by cv IR26 root apices was approximately twice that of cv M101 roots (Table 1).

It has been customarily assumed by plant physiologists that ion absorption occurs through the formation of reversibly-dissociable ion-carrier intermediates in the plasmalemma[1,2,3,14]. The reversible association of Zn and the membrane-bound carrier is considered to occur very rapidly in comparison to its rate of dissociation and the concomitant deposition of Zn into the 'inner space', A further premise inherent to this discussion is the consideration of the Km values as being approximately equal to the dissociation constants of the Zn-carrier complexes. Thus, the reciprocals of the Km values are interpreted herein as an approximation of the affinity of the carriers for Zn. From this viewpoint, root apices from rice cv M101 appear to have an affinity for Zn that is about two-fold greater than that of cv IR26 root apices.

Table 1. Apparent Km and Vmax values for uptake of Zn by excised root apices of rice cultivars M101 and IR26 at pH 5.7 and 26° in the presence of 0.5 m*M* $CaSO_4$. (Data expressed on a 'root fresh weight' basis)

Rice cultivar	Km, m*M*	Vmax (umoles Zn absorbed/ g fr. wt/ 30 min)
M101	0.006	1.45
IR26	0.013	2.87

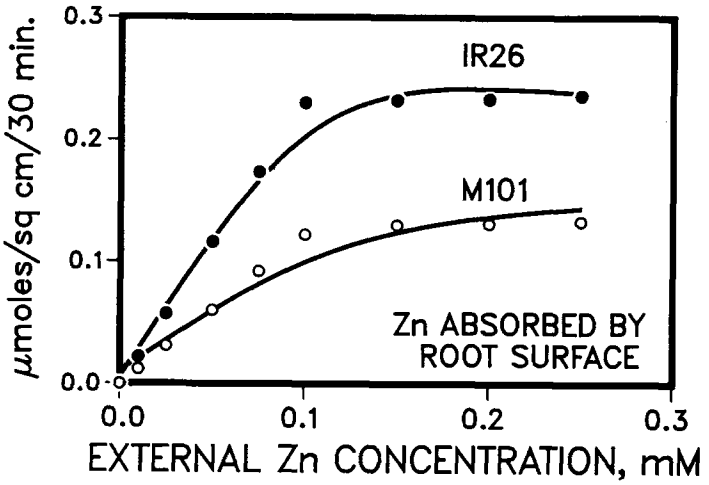

Fig. 3. Uptake of Zn by excised root apices from rice cultivars IR26 and M101 as a function of the external Zn concentration. Data are expressed on a 'unit of root surface area' basis. All conditions same as in Figure 2 otherwise.

Zn uptake as a function of root surface area

The possibility was also considered that the apparent differences in rates of Zn uptake between rice cv M101 and cv IR26 could be attributed to differences in root diameter and thus surface area exposed to the external Zn solution. The rates of Zn absorption were measured as functions of the exogenous Zn concentration, using root apices from each cultivar as described above. However, the data were expressed in terms of 'unit of root surface area' rather than 'root weight' (Fig. 3). The surface areas per gram fresh weight of roots were 12.31 sq cm and 14.84 sq. cm for cvs M101 and IR26, respectively. This difference was not statistically significant.

Roots of Zn-inefficient cv IR26 absorbed Zn at a rate twice that of Zn-efficient cv M101 regardless of whether the data were expressed in terms of root weight (Fig. 2) or surface area (Fig. 3). Kinetic

Table 2. Apparent Km and Vmax values for uptake of Zn by excised root apices of rice cultivars M101 and IR26 at pH 5.7 and 26° in the presence of 0.5 m*M* $CaSO_4$. (Data expressed on a 'unit of root surface area' basis)

Rice cultivar	Km, m*M*	Vmax (umoles Zn absorbed/ sq cm of surface area/30 min
M101	0.029	0.130
IR26	0.051	0.239

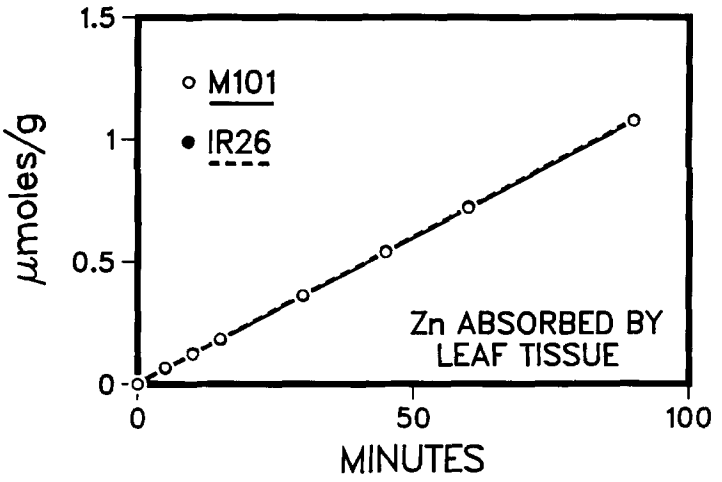

Fig. 4. Active uptake of Zn by leaf blade tissue from rice cultivars IR26 and M101 as a function of time. External solution contained 0.10 m*M* Zn as $ZnSO_4 . 7H_2O$ and 0.50 m*M* $CaSO_4$; pH 5.7 ± 0.2; temperature, 26° ± 1°; desorption, 30 min in 0.50 m*M* $CaSO_4$ at 4°.

constants for uptake per unit of root surface area are shown in Table 2. Both Km and Vmax for cv IR26 are approximately double the respective kinetic parameters for cv M101 (Table 2). The same relationship prevailed between Km and Vmax when Zn uptake was expressed on a root weight basis (Table 1). The relative affinity of cv M101 roots for Zn was likewise double that of cv IR26 roots regardless of the method by which uptake was expressed.

Based upon these data, it is concluded that the difference in Zn uptake rates between cv IR26 and cv M101 cannot be explained as a difference in root surface area between these two cultivars.

Zn uptake by rice leaf tissue

Absorption of Zn from a 0.10 m*M* Zn solution by leaf blade tissue from cv M101 and cv IR26 was linear with time for at least 90 min (Fig. 4). However, there were no statistically significant differences

Table 3. Apparent Km and Vmax values for uptake of Zn by leaf tissue of rice cultivars M101 and IR26 at pH 5.7 and 26° in the presence of 0.5 m*M* $CaSO_4$

Rice cultivar	Km, m*M*	Vmax (umoles Zn absorbed/ g fr. wt/30 min)
M101	0.036	0.395
IR26	0.042	0.338

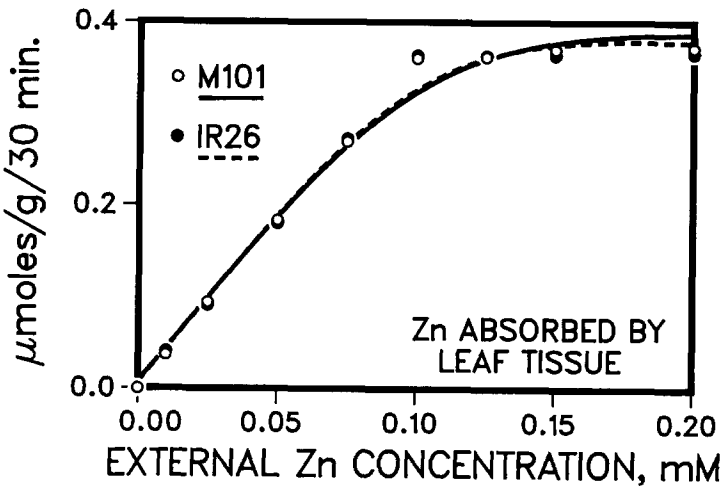

Fig. 5. Uptake of Zn by leaf blade tissue from rice cultivars IR26 and M101 as a function of the external Zn concentration. $CaSO_4$ concentration, 0.50 m*M*; absorption period, 30 min; pH 5.7 ± 0.2; temperature 26° ± 1°; desorption, 30 min in 0.50 m*M* $CaSO_4$ at 4°.

between the rates of Zn uptake by leaf tissue from these two cultivars (Fig. 4). On the other hand, Zn uptake was two-fold greater in cv IR26 roots as compared to those of cv M101 (Fig. 1).

Zinc uptake by leaf blade tissue was then measured as a function of the exogenous Zn concentration over the range of 0.01 m*M* to 0.50 m*M*. Again, there was not a statistically significant difference in the rates of uptake between these two cultivars (Fig. 5). The Zn uptake mechanism in the leaf blade tissue of each rice cultivar was saturated at an external Zn concentration of approximately 0.10 m*M* (Fig. 5). This is the same approximate exogenous Zn concentration at which the Zn uptake system in the excised root apices was saturated (Fig. 2 and 3).

Kinetic constants, Km and Vmax, for Zn uptake by leaf blade tissue from each rice cultivar are given in Table 3. Both parameters were similar for each cultivar. The affinities for Zn of the membrane-bound carriers in the leaf blade tissue, estimated from reciprocals of the Km values, were not statistically significantly different either.

Discussion

It is well known to the world's rice producers that some cultivars of this crop require greater amounts of Zn than do others[5,6,8,9,12,13]. Of the two cultivars used in this study, IR26 is considered to be Zn-inefficient whereas cv M101 is Zn-efficient. That is, cv M101 thrives in hydroponic solutions that would usually be considered to be Zn-deficient; *i.e.*, that contain less than 0.10 mg Zn per liter of solution. This was confirmed in these experiments. Cultivar IR26 plants developed symptoms of Zn deficiency quite readily in hydroponic culture in the greenhouse when Zn was withheld, whereas the contaminant Zn concentration – 0.10 mg Zn/liter – prevented the appearance of characteristic Zn deficiency symptoms on cv M101.

The differential Zn requirement in rice cultivars M101 and IR26 is apparently attributable to differences in affinity of the roots for this micro-nutrient. It is only in excised root apices that Zn rates varied between these two cultivars. There were no differences in Zn uptake rates in leaf blade tissues in these experiments.

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References

1 Bowen J E 1969 Absorption of copper, zinc and manganese by sugarcane leaf tissue. Plant Physiol. 44, 255–261.
2 Bowen J E 1973 Kinetics of zinc absorption by excised roots of two sugarcane clones. Plant and Soil 39, 125–129.
3 Bowen J E and Nissen P 1976 Boron uptake by excised barley roots. I. Uptake into the free space. Plant Physiol. 57, 353–357.
4 Dittmer H J 1937 A quantitative study of the roots and root hairs of winter rye plants. Am. J. Bot. 24, 417–420.
5 Giordano P M and Mortvedt J J 1974 Response of several rice cultivars to zinc. Agron. J. 66, 220–223.
6 Giordano P M, Noggle J C and Mortvedt J J 1974 Zinc uptake by rice as affected by metabolic inhibitors and competing cations. Plant and Soil 41, 637–646.
7 Integrated Software Systems Corp 1983 Tell-a-Graf User's Guide. ISSC, San Diego, Calif.
8 International Rice Research Institute 1977 Annual Report for 1976. pp 97–104.
9 International Rice Research Institute 1978 Annual Report for 1977. pp 124–125.
10 Lineweaver H and Burk D 1936 The determination of enzyme dissociation constants. J. Am. Chem. Soc. 56, 658–666.
11 Ohira K, Ojima K, Saigusa M and Fujiwara A 1975 Studies on the nutrition of rice cell culture II. Microelement requirement and the effects of deficiency. Plant Cell Physiol. 16, 73–81.
12 Ponnamperuma F N 1976 Screening rice for tolerance to mineral stress. *In* Plant Adaptation to Mineral Stress in Problem Soil. Eds M J Wright and S A Ferrari. Cornell Univ. Ithaca, New York. pp 341 1–353.

13 Sadana U S and Takker P N 1983 Effect of calcium and magnesium on ^{65}zinc absorption and translocation in rice seedlings. J. Plant Nutr. 6, 705–715.
14 Schmid W E, Haag H P and Epstein E 1965 Absorption of zinc by excised barley roots. Physiol. Plant. 18, 860–869.
15 Snedecor G W 1948 Statistical Methods Applied to Experiments in Agriculture and Biology. Iowa State College Press, Ames, Iowa. 485 p.
16 Takahashi D T 1968 Zinc deficiency – varietal differences. Hawaiian Sugar Planters' Assoc. Expt. Sta. Ann. Rpt. 1968, 17–18.