High- and Low-Affinity Zinc Transport Systems and Their Possible Role in Zinc Efficiency in Bread Wheat[1]

Gokhan Hacisalihoglu, Jonathan J. Hart*, and Leon V. Kochian

United States Plant, Soil, and Nutrition Laboratory, United States Department of Agriculture–Agricultural Research Service, Cornell University, Ithaca, New York 14853

There is considerable variability among wheat (*Triticum aestivum* L.) cultivars in their ability to grow and yield well in soils that contain very low levels of available Zn. The physiological basis for this tolerance, termed Zn efficiency, is unknown. We investigated the possible role of Zn^{2+} influx across the root cell plasma membrane in conferring Zn efficiency by measuring short-term $^{65}Zn^{2+}$ uptake in two contrasting wheat cultivars, Zn-efficient cv Dagdas and Zn-inefficient cv BDME-10. Plants were grown hydroponically under sufficient and deficient Zn levels, and uptake of $^{65}Zn^{2+}$ was measured over a wide range of Zn activities (0.1 nM–80 μM). Under low-Zn conditions, cv BDME-10 displayed more severe Zn deficiency symptoms than cv Dagdas. Uptake experiments revealed the presence of two separate Zn transport systems mediating high- and low-affinity Zn influx. The low-affinity system showed apparent K_m values similar to those previously reported for wheat (2–5 μM). Using chelate buffered solutions to quantify Zn^{2+} influx in the nanomolar activity range, we uncovered the existence of a second, high-affinity Zn transport system with apparent K_m values in the range of 0.6 to 2 nM. Because it functions in the range of the low available Zn levels found in most soils, this novel high-affinity uptake system is likely to be the predominant Zn^{2+} uptake system. Zn^{2+} uptake was similar for cv Dagdas and cv BDME-10 over both the high- and low-affinity Zn^{2+} activity ranges, indicating that root Zn^{2+} influx does not play a significant role in Zn efficiency.

Zn is an essential element for plants and other organisms and is involved in many cellular processes, including activation of enzymes, protein synthesis, and membrane stability (Welch et al., 1982; Marschner, 1986).

Zn deficiency, defined as the condition in which insufficient Zn is available for optimal growth, may cause dramatic reductions in crop yield and quality. Zn deficiency has become a serious agricultural problem. It is associated with high-pH calcareous soils and sandy, highly leached soils, which cause reduced Zn availability and low total Zn content, respectively (Swietlik, 1989). Zn deficiency is considered to be one of the most widespread micronutrient problems for crops (Cakmak et al., 1999), occurring in 30% of the world's soils (Sillanpaa, 1982). It is unfortunate that the use of Zn fertilizers does not completely alleviate Zn deficiency due to factors such as subsoil constraints, topsoil drying, or disease interactions (Graham and Rengel, 1993). Moreover, Zn fertilizers may be unavailable or unaffordable in developing countries. Because of the widespread problems of Zn deficiency and difficulties in alleviating it via fertilization, a promising alternative may be the identification of Zn efficient genotypes. Zn efficiency is defined as the ability of a plant to maintain good growth and yield on Zn-limited soils (Graham, 1984). A number of plant species exhibit significant intraspecific variation in Zn efficiency, which appears to be under genetic control. Zn deficiency in wheat (*Triticum aestivum* L.) occurs in several parts of the world and wheat genotypes exhibit a great diversity in their ability to grow on Zn-deficient soils (Graham et al., 1992).

Despite the potential agricultural and economical importance of the Zn efficiency trait, physiological mechanisms of differential Zn efficiency remain unknown. It is important to understand these mechanisms to develop improved cultivars for low-input wheat production on low Zn soils in developing countries. Several mechanisms have been proposed to explain Zn efficiency in crop plants, including increased Zn uptake, increased Zn bioavailability in the rhizosphere due to release of root exudates, and more efficient internal Zn use (for review, see Rengel, 1999).

Recent research investigating potential mechanisms of Zn efficiency have yielded somewhat equivocal results. Rengel and Graham (1995) found no correlation between Zn uptake rate and dry matter production in several wheat genotypes. Cakmak et al. (1997) found similar results with Turkish wheat cultivars under different growing conditions and concluded that plant tissue Zn concentration is not a dependable parameter for evaluating differential Zn efficiency among genotypes. More recently, Erenoglu et al. (1999) compared Zn uptake in rye and wheat and found that Zn deficiency stimulated Zn uptake in both species, but no positive correlation was found between efficiency and uptake rate in bread wheat. In another study, Rengel and Wheal (1997) examined

[1] This work was supported by the Republic of Turkey (graduate fellowship to G.H.).

* Corresponding author; e-mail jjh16@cornell.edu; fax 607–255–1132.

Zn uptake kinetics of bread wheat cultivars and found that efficient cultivars had higher net uptake rate (I_{max}) but similar apparent K_m values. Most of these studies either compared different plant species or used high-Zn concentrations, which do not simulate the Zn concentrations of soil solution. Also, all of these studies measured Zn uptake over a long time period, which does not necessarily reflect the ability of roots to absorb Zn from the soil under low-Zn conditions. Long-term Zn uptake in these studies can reflect and be influenced by a number of factors including Zn compartmentation in roots, as well as translocation and use in shoots. The experimental protocols for measuring unidirectional Zn influx into roots are well established. Hart et al. (1998), in their studies of concentration-dependent kinetics of root Zn uptake in two different wheat species, found that a Zn-efficient bread wheat cultivar exhibited higher Zn influx rates than a Zn-inefficient durum wheat cultivar at low-solution Zn^{2+} activities (0.01–200 nM).

The objectives of the present study were to: (a) examine the concentration-dependent kinetics of Zn^{2+} influx into roots of two bread wheat cultivars (with contrasting Zn efficiency) to determine whether differential Zn efficiency is due to differences in root Zn uptake; and (b) investigate the kinetics of Zn uptake in detail at both low-solution Zn activities that reflect soil solution Zn^{2+} levels (0–160 nM), as well as at higher Zn concentrations used in previous studies (0–75 μM). It has been shown that graminaceous monocot roots may be able to absorb both Zn^{2+} and Zn-phytosiderophore chelates (von Wiren et al., 1996). However, in this study, only Zn^{2+} uptake was examined. The results demonstrated the existence of both high- and low-affinity transport systems in wheat roots and a lack of correlation between Zn efficiency and root Zn uptake in bread wheat.

RESULTS

Evaluation of Zn Efficiency

Results from growth experiments in chelate-buffered solution culture for evaluation of Zn efficiency are shown in Table I. Zn efficiency was evaluated by the influence of low-solution Zn^{2+} activity on root and shoot elongation and dry weight and on root and shoot Zn concentrations. When grown in a low-Zn^{2+} activity (0.48 pM) nutrient solution, the efficient cv Dagdas had higher root and shoot dry weight, longer roots and leaves, but lower root and shoot Zn concentrations. At the higher Zn^{2+} activities (0.96–58.0 pM), there were no significant differences detected between efficient cv Dagdas and inefficient cv BDME-10 for tissue Zn^{2+} concentrations and root and shoot growth parameters. Moreover, cv BDME-10 exhibited significantly greater visual symptoms of Zn deficiency (stunting, reduced tillering, chlorosis, and necrosis of middle leaves) than the Zn-efficient cv Dagdas at low-Zn activities. Thus, using chelate-buffered techniques, we were able to devise a hydroponic culture that allowed us to assess Zn inefficiency and efficiency in these two bread wheat cultivars. As it was necessary to use seedlings considerably younger (10 d old) than the 21-d-old seedlings used for these growth experiments to fit the root systems into the Plexiglas wells of the uptake system, the low-Zn grown seedlings for root Zn uptake studies were grown on a lower solution Zn^{2+} activity (0.048 pM) to ensure that mild Zn deficiency symptoms were

Table I. *Effect of varying nutrient solution Zn activity on root and shoot elongation, dry wt, and tissue Zn concentrations in 21-d-old cv Dagdas and cv BDME-10 wheat seedlings*

Data represent means and SE (in parentheses) of four replications.

Cultivar and Measured Parameter	Zn^{2+} Activity in Nutrient Solution				
	0.48 pM	0.96 pM	2.9 pM	4.88 pM	58 pM
Dry wt of roots and shoots (mg)					
cv BDME-10 roots	16 (4)	21 (2)	33 (7)	40 (3)	74 (6)
cv Dagdas roots	47 (6)	31 (10)	44 (20)	60 (10)	76 (5)
cv BDME-10 shoots	34 (8)	56 (6)	90 (10)	140 (20)	297 (10)
cv Dagdas shoots	71 (5)	66 (20)	89 (30)	140 (30)	308 (20)
Tissue Zn concentrations of roots and shoots (μg/g)					
cv BDME-10 roots	50.6 (8.2)	36.1 (3.9)	32.2 (6.4)	31.2 (0.8)	49.7 (1.8)
cv Dagdas roots	25.5 (4.8)	29.5 (7.3)	29.2 (8.7)	28.4 (5.4)	54.3 (1.4)
cv BDME-10 shoots	18.6 (1.9)	10.1 (0.9)	10.3 (1.0)	10.8 (1.8)	19.7 (0.4)
cv Dagdas shoots	10.6 (1.2)	12.6 (1.0)	12.6 (4.0)	10.7 (0.8)	20.6 (0.6)
Length of primary roots and leaves (mm)					
cv BDME-10 roots	348 (22)	396 (19)	369 (17)	412 (6)	378 (14)
cv Dagdas roots	584 (29)	466 (39)	370 (33)	594 (30)	478 (42)
cv BDME-10 shoots	159 (22)	198 (22)	267 (21)	305 (4)	343 (11)
cv Dagdas shoots	197 (14)	216 (17)	319 (12)	286 (15)	336 (12)

observed in Zn-inefficient cv BDME-10. A somewhat higher Zn^{2+} activity (147 pM) was used for growth of Zn-sufficient plants.

Kinetics of $^{65}Zn^{2+}$-Influx into Wheat Roots

Low-Affinity $^{65}Zn^{2+}$ Uptake

The concentration-dependent kinetics of root Zn^{2+} influx were studied in Zn-sufficient and -deficient seedlings of cv Dagdas and cv BDME-10 wheat over a wide range of external Zn^{2+} activities (0.1 nM–80 μM). This was done by quantifying Zn^{2+} influx over two different concentration ranges: a high-concentration range (low-affinity uptake, 0.4–80 μM) to compare our results with previously published studies, and a low-concentration range (high-affinity uptake, 0.1–160 nM) that is more representative of soil solution Zn^{2+} activities.

Low-affinity Zn uptake in Zn-sufficient and -deficient seedlings of Zn-inefficient cv BDME-10 and -efficient cv Dagdas are depicted in Figures 1 and 2. In all cases, the kinetics of root Zn^{2+} influx were complex, non-saturating curves that could be resolved graphically into saturable and linear components. Similar complex kinetics for root Zn^{2+} influx were previously obtained in wheat (Hart et al., 1998) and *Thlaspi caerulescens* (Lasat et al., 1996). In both cases it was shown that the linear "uptake" component was actually cell wall-bound ^{65}Zn remaining in the root apoplasm after desorption, whereas the saturable component was bona fide Zn^{2+} influx across the root cell plasma membrane.

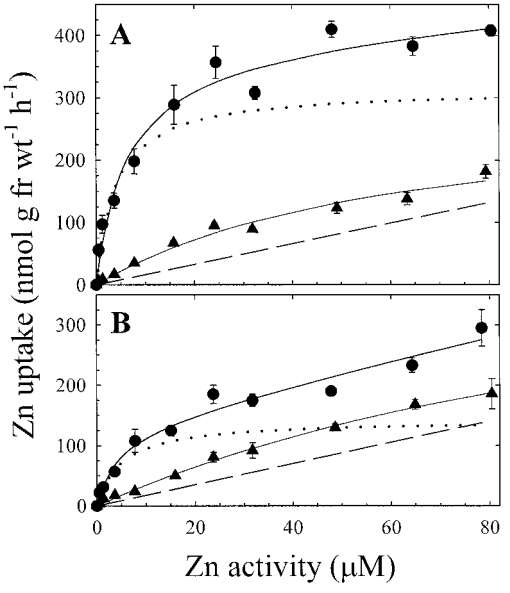

Figure 1. Concentration-dependent kinetics for low-affinity root $^{65}Zn^{2+}$ influx in Zn-sufficient seedlings of Zn-inefficient cv BDME-10 (A) and Zn-efficient cv Dagdas (B) wheat at high (0.4–80 μM) Zn concentrations. The linear (dashed line) and saturable (dotted line) components were derived from the experimental data (●) by computing the linear component from the regression line plotted through high-concentration points and subtracting this contribution from a curve fit to the experimental data. Error bars do not extend outside some data points. Error bars represent means (n = 4) ± SE. ▲, Zn uptake at 2°C.

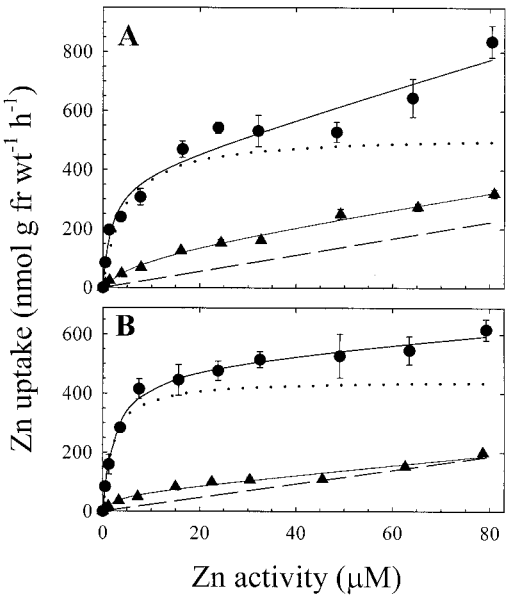

Figure 2. Concentration-dependent kinetics for low-affinity root $^{65}Zn^{2+}$ influx in Zn-deficient cv BDME-10 (A) and cv Dagdas (B) wheat seedlings at high-Zn concentrations. The linear (dashed line) and saturable (dotted line) components were derived as described in Figure 1. Error bars do not extend outside some data points. Error bars represent means (n = 4) ± SE. ▲, Zn uptake at 2°C.

We conducted a similar analysis here to determine the nature of the two components. Exposure of roots to low temperature (2°C) to inhibit metabolically-coupled processes selectively abolished saturable Zn uptake, leaving the linear component unaffected (Figs. 1 and 2). In addition, the concentration-dependent kinetics of $^{65}Zn^{2+}$ binding to morphologically intact root cell wall preparations was conducted. These root cell wall preparations were prepared by treating roots with chloroform-methanol to dissolve away lipidic components in the root (Hart et al., 1998). When our protocols for the $^{65}Zn^{2+}$ uptake experiments were repeated with these root cell wall preparations, they also yielded linear kinetics that were nearly identical to the linear components graphically derived in Figures 1 and 2 (data not shown). These findings strongly indicated that only the saturable component represented true low-affinity Zn^{2+} uptake across the root cell plasma membrane.

Several points should be raised based on the data in Figures 1 and 2. First, it is clear that the imposition of Zn deficiency greatly stimulated low-affinity Zn uptake in both cultivars. As depicted in Table II, Zn deficiency caused a 1.7- to 3-fold stimulation in the V_{max} for Zn uptake, while having little effect on the apparent K_m. The second point is that there were no apparent differences in root Zn uptake between the

Table II. *Kinetic parameters for root Zn^{2+} influx in Zn-sufficient [(+)Zn] and Zn-deficient [(−)Zn] grown cv Dagdas and cv BDME-10 wheat seedlings*

Values for V_{max} (maximal Zn influx) and apparent K_m (Michaelis constant) were obtained by fitting a hyperbolic curve to the calculated saturable Zn influx data derived from plots of root Zn influx rate versus uptake solution Zn^{2+} activity. Numbers in parentheses represent SE of regression coefficient estimates.

	V_{max}				Apparent K_m			
	(−) Zn Grown Plants		(+) Zn Grown Plants		(−) Zn Grown Plants		(+) Zn Grown Plants	
	cv BDME-10	cv Dagdas	cv BDME-10	cv Dagdas	cv BDME-10	cv Dagdas	cv BDME-10	cv Dagdas
	nmol g fresh wt^{-1} h^{-1}				*μM*			
Low-affinity Zn^{2+} uptake system	521 (38)	446 (11)	294 (17)	143 (11)	4.1 (1.5)	1.9 (0.3)	3.4 (1.1)	4.9 (1.7)
					nM			
High-affinity Zn^{2+} uptake system	30.9 (2.2)	10.9 (1.5)	9.7 (1.0)	9.0 (0.6)	2.3 (1.0)	0.6 (0.6)	0.7 (0.5)	1.2 (0.5)

two cultivars that could account for differences in Zn efficiency, a point that will be considered in more detail in the "Discussion."

High-Affinity $^{65}Zn^{2+}$ Uptake

A chelate buffer approach was used to quantify root Zn^{2+} influx over a range of low-Zn activities more representative of Zn^{2+} activities in the soil solution. As shown in Figures 3 and 4, these again yielded complex kinetics that could be graphically resolved into saturable and linear components. However, as shown in Figure 5, when the kinetics of Zn^{2+} influx were quantified at low temperature (2°C), both saturable and linear Zn uptake components were abolished, suggesting that both represent true Zn uptake. Thus, we computed the contribution from low-affinity Zn uptake over this low-Zn concentration range (0.1–160 nM) to the total uptake presented in Figures 3 and 4. It was found that the contribution to high-affinity uptake over this nanomolar Zn^{2+} activity range by the low-affinity transporter yielded linear transport kinetics that were identical to the linear components graphically determined in Figures 3 and 4. When this contribution from low-affinity Zn uptake was subtracted from total uptake, a separate high-affinity Zn^{2+} uptake system was revealed with an apparent K_m value for Zn^{2+} ranging from 0.6 to 2.3 nM in Zn-sufficient and -deficient cv Dagdas and cv BDME-10 wheat (Table II). As was shown previously for the low-affinity Zn transporter, there were no obvious differences in apparent K_m or V_{max} values for high-affinity uptake between the two cultivars that could account for the differences in Zn efficiency. It is interesting to note that Zn deficiency did stimulate high-affinity Zn uptake, but only in the Zn-inefficient cv BDME-10 (Table II).

DISCUSSION

Zn Efficiency of Bread Wheat Cultivars

We first used chelate buffer techniques to design hydroponic growth solutions in which the free Zn^{2+} activity was controlled at sufficiently low levels to observe significant differences in the ability to resist Zn deficiency in bread wheat cultivars previously reported to be Zn efficient (cv Dagdas) and inefficient (cv BDME-10; Erenoglu et al., 1999). We found that at the lowest hydroponic solution Zn^{2+} activities used (below 0.5 pM), cv BDME-10 showed significant visual symptoms of Zn deficiency after

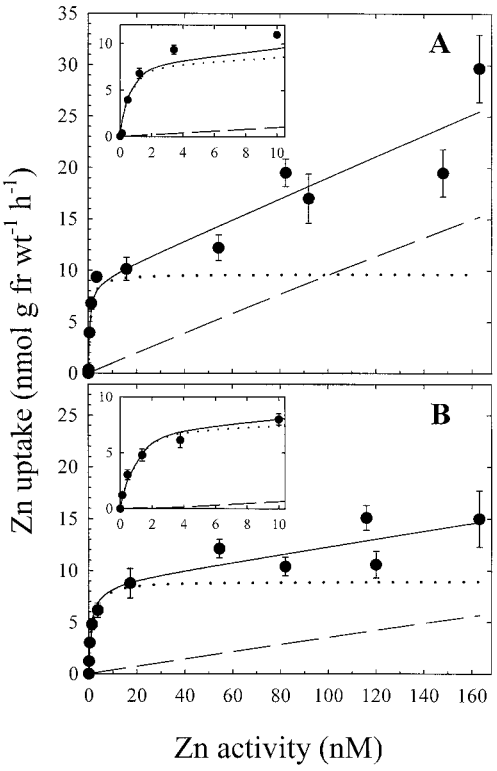

Figure 3. Concentration-dependent kinetics for high-affinity root $^{65}Zn^{2+}$ influx in Zn-sufficient cv BDME-10 (A) and cv Dagdas (B) wheat at low Zn concentrations (0.1–160 nM). Inserts depict the kinetics of $^{65}Zn^{2+}$ influx from 0.1 to 10 nM Zn^{2+}. The kinetics have been graphically resolved into linear (dashed line) and saturable (dotted line) components as described in Figure 1. Error bars do not extend outside some data points. Error bars represent means (n = 4) ± SE.

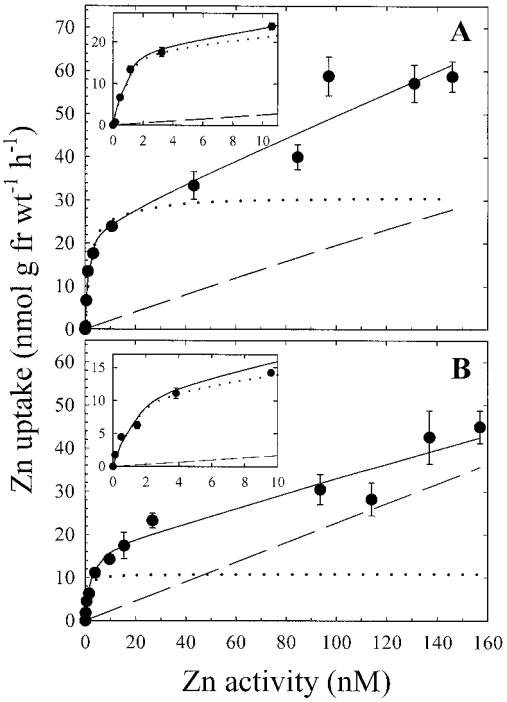

Figure 4. Concentration-dependent kinetics for high-affinity root $^{65}Zn^{2+}$ influx in Zn-deficient cv BDME-10 (A) and cv Dagdas (B) wheat at low Zn concentrations (0.1–160 nM). Inserts depict the kinetics of $^{65}Zn^{2+}$ influx from 0.1 to 10 nM Zn^{2+}. The kinetics have been graphically resolved into linear (dashed line) and saturable (dotted line) components as described in Figure 1. Error bars do not extend outside some data points. Error bars represent means (n = 4) ± SE.

21 d of growth, whereas cv Dagdas did not exhibit Zn deficiency. Furthermore, at these low-Zn^{2+} activities, cv BDME-10 wheat seedlings exhibited greatly reduced root and shoot growth, both in terms of dry weight production and root and leaf expansion (Table I). At the higher Zn^{2+} activities, there were no dramatic differences in shoot and root biomass between the two wheat cultivars. However, at most hydroponic solution Zn^{2+}activities, the Zn-efficient cv Dagdas exhibited a moderate increase in root length compared with the Zn-inefficient cv BDME-10 (Table I), indicating that efficient plants may be able to access and explore a greater soil volume and thus absorb more Zn from the soil. A similar finding was reported by Dong et al. (1995) where the Zn-efficient wheat genotypes developed longer and thinner roots than inefficient genotypes in Zn-deficient soil. It is interesting to note that for both Zn-efficient cv Dagdas and inefficient cv BDME-10, root and shoot Zn concentrations were similar, except at the lowest Zn^{2+} activity where it appears that the growth reduction caused by Zn deficiency in cv BDME-10 may have resulted in moderate increases in root and shoot Zn concentrations. These findings, particularly the observation that shoot Zn concentrations did not differ greatly even when cv BDME-10 was exhibiting fairly severe Zn deficiency symptoms, suggest that absorption of Zn by roots does not play a major role in differences in Zn efficiency. These results confirm an earlier report by Cakmak et al. (1997) of similar root and shoot Zn^{2+} concentrations for Zn-efficient and -inefficient cultivars.

Kinetics of $^{65}Zn^{2+}$ Influx in Wheat Roots

Although there have been several previous comparative studies of root Zn absorption by Zn-efficient and -inefficient wheat cultivars, in none of these studies was uptake studied at sufficiently low and physiologically relevant Zn^{2+} activities (Bowen, 1986; Mullins and Sommers, 1986; Wheal and Rengel, 1997; Erenoglu et al., 1999). Furthermore, in none of the previous studies was a rigorous quantification of root Zn^{2+} influx carried out. Radiotracer flux techniques that we had developed previously for quantifying root Zn^{2+} influx (Lasat et al., 1996; Cohen et al., 1998; Hart et al., 1998) were used in this study. As in the previous studies, the concentration-dependent kinetics for Zn influx over a broad concentration range (0.4–80 μM Zn) yielded smooth non-saturating curves for Zn^{2+} uptake that could be readily dissected into linear and hyperbolic (saturable) compo-

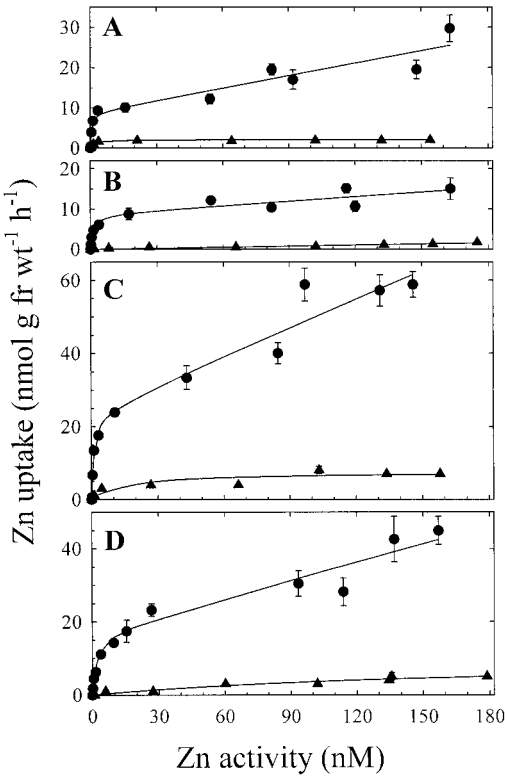

Figure 5. The influence of low temperature (2°C) on high-affinity root $^{65}Zn^{2+}$ influx in Zn-sufficient cv BDME-10 (A) and cv Dagdas (B) wheat seedlings and Zn-deficient cv BDME-10 (C) and cv Dagdas (D) wheat seedlings. Data points and bars represent means and SE values of four replicates. Error bars do not extend outside some data points. Zn uptake at 2°C (▲) is compared with uptake at 23°C (●) in A through D.

nents (Figs. 1 and 2). Previous work from our laboratory using roots of other species and/or other wheat cultivars showed that the linear component of apparent Zn^{2+} uptake was actually root cell wall-bound $^{65}Zn^{2+}$ that remained after desorption (Lasat et al., 1996; Cohen et al., 1998; Hart et al., 1998). Results from experiments presented here for Zn^{2+} uptake from solutions containing micromolar Zn also showed this to be the case (Figs. 1 and 2). Thus, after correction for this linear kinetic component, true Zn influx into root cells was described by uptake systems following Michaelis-Menten kinetics.

Identification of Two Separate Uptake Systems for Zn: Low- and High-Affinity Zn Transport

Based on the kinetic flux analysis conducted in this study, it appears that in the micromolar Zn concentration range, root Zn^{2+} influx in both wheat cultivars can be described by a single low-affinity transport system with an apparent K_m in the 2 to 5 μM range (Figs. 1 and 2; Table II). Similar low-affinity root Zn transport systems have been described previously in wheat (Hart et al., 1998) and the Zn hyperaccumulator, *T. caerulescens* (Lasat et al., 1996). Furthermore, the recent cloning of Zn^{2+} transporter genes in Arabidopsis (*ZIP1-4*, Grotz et al., 1998) and *T. caerulescens* (*ZNT1*; Pence et al., 2000) showed similar transport kinetics when expressed in yeast. In both cases, the kinetics of Zn uptake were characterized as Michaelis-Menten, with apparent K_m values in the low micromolar range.

Characterization of this low-affinity Zn transporter in Zn-deficient and -sufficient seedlings of the two wheat cultivars revealed several features of importance to plant Zn nutrition and Zn efficiency. First, as illustrated in Figures 1 and 2 and Table II, imposition of Zn deficiency elicited a strong stimulation in the V_{max} for low-affinity Zn^{2+} influx with little effect on the apparent K_m. This is consistent with up-regulation of expression of the Zn transporter by decreasing plant Zn status. This type of up-regulation has been observed based on northern analysis of the zinc and iron-inducible protein family of Arabidopsis Zn transporters as well as the ZNT1 Zn transporter in *Thlaspi* (Grotz et al., 1998; Pence et al., 2000). The second point to be made is that low-affinity root Zn uptake does not appear to play a role in the differences in Zn efficiency exhibited in these two wheat cultivars. Although the apparent K_m value for the low-affinity system in Zn efficient cv Dagdas exhibited a small decrease in K_m, (from 5 to 2 μM; Table II), it is likely this decrease is not physiologically relevant, as it does not significantly impact Zn uptake at Zn levels normally found in agricultural soils. Rather, a high-affinity Zn^{2+} uptake system (discussed below) would appear to function as the primary means of Zn^{2+} uptake under conditions of low available soil Zn^{2+}.

All of the previous studies of root Zn uptake with crop plants have focused on low-affinity Zn transport that operates at relatively high soil Zn concentrations (micromolar Zn). However, the activity of soil solution Zn^{2+}, particularly for plants growing on low Zn soils is considerably lower than the micromolar levels used in previous studies (Reid et al., 1996). Therefore, we used chelate buffered radiolabeled solutions to quantify unidirectional root Zn^{2+} influx in the nanomolar activity range. As shown in Figures 3 and 4, this allowed us to uncover the existence of a high-affinity Zn transporter, with an apparent K_m for Zn^{2+} in the low nanomolar range. These findings provide the first evidence, to our knowledge, for two separate uptake systems for Zn mediating high- and low-affinity transport in higher plants. Our initial kinetic analyses of root Zn influx in the nanomolar Zn concentration range suggested that high-affinity Zn uptake was complex, as we saw previously for low-affinity uptake (Figs. 3 and 4). However, unlike low-affinity transport, low temperature abolished both saturable and linear Zn uptake, suggesting both represent true Zn transport into root cells. Further analysis of these kinetic data indicated that the linear component for high-affinity uptake was actually the small contribution to the total uptake by the low-affinity transporter operating in the nanomolar concentration range. When this was subtracted from total uptake, a high-affinity transporter following Michaelis-Menten kinetics was revealed with apparent K_m values for Zn^{2+} ranging from 0.7 to 2.3 nM (Table II).

This high-affinity uptake system in wheat roots is likely to represent the predominant Zn^{2+}-uptake system in soils with low available Zn levels. As seen in Figures 3 and 4, at soil Zn^{2+} activities below approximately 10 nM, the high-affinity system should function to take up almost all available Zn^{2+}, whereas the low-affinity system would account for very little Zn^{2+} uptake (compare the dashed line representing the low-affinity component with the saturable curve of the high-affinity system). In soils with available Zn^{2+} activities greater than 10 nM, the low-affinity system should assume a more important role in Zn^{2+} uptake. Therefore, the high-affinity Zn^{2+} uptake system would appear to be a critical determinant of the ability of plants to acquire Zn, particularly from soils at the low end of the 1 nM to 1 μM Zn^{2+} range reported for agricultural soils (Welch, 1995).

As we observed for the low-affinity Zn transporter, the V_{max} for this high-affinity system was stimulated by the imposition of Zn deficiency, although in this case the transporter was stimulated much more in roots of Zn-inefficient cv BDME-10 (Table II). Also, as was the case for low-affinity Zn uptake, there was no correlation between uptake via this system and differences in Zn efficiency between the two wheat cultivars.

Zn^{2+} Uptake and Zn Efficiency

In this study, we have examined the role of root Zn^{2+} influx in differential Zn efficiency in contrasting bread wheat cultivars. Recently, Erenoglu et al. (1999) presented data suggesting that the differential Zn efficiency expressed in the bread wheat genotypes cv Dagdas, cv BDME-10, and cv Bezostaja was not connected to their Zn uptake capacity. Results from our studies show conclusively that root Zn^{2+} uptake was similar for efficient cv Dagdas and inefficient cv BDME-10 over a wide range of plant Zn status and Zn^{2+} activities in the uptake solution. This conclusion differs from that of Hart et al. (1998), who found a correlation between Zn^{2+} uptake and Zn efficiency in a study comparing bread wheat and durum wheat cultivars. The differential uptake kinetics measured in that study may be related to genetic differences between the two different wheat species.

The present study serves as a springboard for further investigations of mechanisms of Zn efficiency in wheat. The findings presented here suggest that differences in Zn compartmentation or use in the shoot may play a critical role in the underlying mechanisms of efficiency. Therefore, future research will include compartmentation studies to determine if the inefficient wheat cultivar sequesters a larger fraction of the shoot Zn in the vacuole, where it might be unavailable for use in Zn-requiring physiological processes and investigations to determine if Zn-binding ligands might be involved in lowering the concentrations of physiologically "active" Zn in the cytosol.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The two bread wheat (*Triticum aestivum*) cultivars used in these experiments were cv BDME-10 and cv Dagdas. Both of these cultivars are widely grown in Turkey and were selected for their relative Zn requirements. cv BDME-10 requires considerably higher levels of soil Zn than cv Dagdas, and these cultivars have been classified Zn-inefficient and -efficient, respectively (Erenoglu et al., 1999). Seeds of cv BDME-10 and cv Dagdas were surface sterilized in 0.5% (v/v) NaOCl for 20 min, rinsed, and germinated on filter paper in the dark. After 24 h, uniform seedlings were transferred to black polyethylene cups with mesh bottoms and covered with black polyethylene beads. Cups were positioned above nutrient solution in holes of light-sealed tops of 5-L polyethylene pots fitted with aeration tubes. The pots were filled with a chelate-buffered solution prepared in 12 MΩ de-ionized water and containing 1 mM KNO_3, 1 mM $Ca(NO_3)_2$, 0.05 mM $NH_4H_2PO_4$, 0.25 mM $MgSO_4$, 0.1 mM NH_4NO_3, 50 μM KCl, 12.5 μM H_3BO_3, 0.1 μM H_2MoO_4, 0.1 μM $NiSO_4$, 0.4 μM $MnSO_4$, 1.6 μM $CuSO_4$, 96 μM $Fe(NO_3)_3$, 118 μM H_3HEDTA, and 2 mM MES [2-(*N*-morpholino)ethanesulfonic acid], pH 6.0. $Fe(NO_3)_3$-H_3HEDTA and $ZnSO_4$-H_3HEDTA were prepared separately before addition to nutrient solutions. Excess HEDTA was used to buffer the metal activities of micronutrients. The free activities of all components in the solution were calculated using the chemical speciation program GEOCHEM-PC (Parker et al., 1995). Plants used in uptake experiments were grown in a controlled-environment growth chamber with a 400 to 500 μmol m^{-2} s^{-1} photon flux density, 20/15°C (16/8 h) day/night temperature regime.

Analysis of Zn-Deficiency Stress

To determine the effects of Zn deficiency, cv Dagdas and cv BDME-10 plants were grown as described above, in nutrient solutions containing one of five Zn concentrations: 0.05, 0.1, 0.3, 0.5, and 5 μM. Free Zn^{2+} activities predicted by GEOCHEM-PC (Parker et al., 1995) were 0.48, 0.96, 2.90, 4.88, and 58.0 pM Zn^{2+}, respectively. Twenty-one-d-old seedlings were harvested, rinsed in 18 MΩ water, blotted dry, placed in coin envelopes, and oven-dried at 65°C for 4 d. Dried shoots and roots were weighed and digested in concentrated HNO_3 overnight at 120°C. Samples were then dissolved in HNO_3:$HClO_4$ (1:1, v/v) at 220°C, resuspended in 5% (v/v) HNO_3 and analyzed for elemental composition via simultaneous inductively coupled argon-plasma emission spectrometry (ICAP 61E trace analyzer, Thermo-Jarrel Ashe, Franklin, MA).

Root $^{65}Zn^{2+}$-Influx Experiments

Plants used for $^{65}Zn^{2+}$ uptake experiments were grown under either Zn-sufficient (147 pM Zn^{2+}) or Zn-deficient (0.05 pM Zn^{2+}) conditions. Intact 10-d-old wheat seedlings were removed from nutrient solution, the roots rinsed in 18 MΩ purity water for 2 min, and then placed in 5-L pots containing pretreatment solution (2 mM MES-Tris, pH 6.0, 0.2 mM $CaSO_4$, 12.5 μM H_3BO_3, 0.15 nM $ZnSO_4$) for 30 min. A custom-built Plexiglas uptake apparatus previously described (Hart et al., 1992) was used for all uptake experiments. Wells of the uptake system were filled with 60 mL of uptake solution consisting of 5 mM MES-Tris, pH 6.0, 0.2 mM $CaSO_4$, and 12.5 μM H_3BO_3, 0.4 μCi $^{65}Zn^{2+}$, and varying concentrations of non-radiolabeled $ZnSO_4$ and EDTA to yield the desired total Zn^{2+} activity (0–160 nM Zn^{2+} for low-concentration range and 0–75 μM Zn^{2+} for high-concentration range). A 1-mL aliquot of uptake solution was removed as an internal standard. Uptake was initiated by gently inserting the roots of intact seedlings into the wells. Separate experiments showed that Zn^{2+} accumulation increased linearly over 90 min, so 20 min was chosen as an appropriate time period to assess unidirectional Zn^{2+} influx. At the end of the 20-min uptake period, a second 1-mL aliquot of the uptake solution was taken to determine the amount of substrate ($^{65}Zn^{2+}$) depletion. Depletion was measured to ensure that roots were exposed to solutions with stable Zn^{2+} concentrations. The uptake solution in the wells was then removed by vacuum withdrawal and replaced with ice-cold (2°C) desorption solution (5 mM MES-Tris, pH 6.0, 5 mM $CaSO_4$, 100 μM $ZnSO_4$). After two

7.5-min desorption periods (15 min total desorption), seedlings were removed from wells, roots were blotted with damp paper towels, excised, and weighed. ^{65}Zn taken up by excised roots was directly measured via γ detection using a γ counter (Auto-Gamma 5530, Packard, Meriden, CT). Experiments were replicated at least two times.

ACKNOWLEDGMENT

We would like to thank Dr. I. Cakmak of Cukurova University, Turkey, for providing the wheat seed used in this study and for many helpful discussions.

Received April 28, 2000; modified August 4, 2000; accepted September 4, 2000.

LITERATURE CITED

Bowen JE (1986) Kinetics of zinc uptake by two rice cultivars. Plant Soil **94:** 99–107

Cakmak I, Ekiz H, Yilmaz A, Torun B, Koleli N, Gultekin I, Alkan A, Eker S (1997) Differential response of rye, triticale, bread and durum wheats to zinc deficiency in calcareous soils. Plant Soil **188:** 1–10

Cakmak I, Kalayci M, Ekiz H, Braun HJ, Kilinc Y, Yilmaz A (1999) Zn deficiency as a practical problem in plant and human nutrition in Turkey: a NATO-Science for stability project. Field Crop Res **60:** 175–188

Cohen CK, Fox TC, Garvin DF, Kochian LV (1998) The role of iron deficiency stress responses in stimulating heavy-metal transport in plants. Plant Physiol **116:** 1063–1072

Dong B, Rengel Z, Graham RD (1995) Root Morphology of wheat genotypes differing in Zn efficiency. J Plant Nutr **18:** 2761–2773

Erenoglu B, Cakmak I, Romheld V, Derici R, Rengel Z (1999) Uptake of zinc by rye, bread wheat and durum wheat cultivars differing in zinc efficiency. Plant Soil **209:** 245–252

Graham RD (1984) Breeding for nutritional characteristics in cereals. Adv Plant Nutr **1:** 57–102

Graham RD, Ascher JS, Hynes SC (1992) Selecting zinc-efficient cereal genotypes for soils of low zinc status. Plant Soil **146:** 246–250

Graham RD, Rengel Z (1993) Genotypic variation in Zn uptake and utilization by plants. *In* AD Robson, ed, Zinc in Soils and Plants. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 107–114

Grotz N, Fox T, Connolly EL, Park W, Eide D (1998) Identification of a family of zinc transporter genes from *Arabidopsis* that respond to zinc deficiency. Proc Natl Acad Sci USA **95:** 7220–7224

Hart JJ, DiTomaso JM, Linscott DL, Kochian LV (1992) Characterization of the transport and cellular compartmentation of paraquat in root of intact maize seedlings. Pestic Biochem Physiol **43:** 212–222

Hart JJ, Norvell WA, Welch RM, Sullivan LA, Kochian LV (1998) Characterization of zinc uptake, binding, and translocation of bread and durum wheat cultivars. Plant Physiol **118:** 219–226

Lasat MM, Baker AJ, Kochian LV (1996) Physiological characterization of root Zn^{2+} absorption and translocation to shoots in Zn hyperaccumulator and nonaccumulator species of *Thlaspi*. Plant Physiol **112:** 1715–1722

Marschner H (1986) Mineral Nutrition of Higher Plants. Academic Press, New York

Mullins GL, Sommers LE (1986) Cadmium and zinc influx characteristics by intact corn seedlings. Plant Soil **96:** 153–164

Parker DR, Norvell WA, Chaney RL (1995) GEOCHEM-PC: a chemical speciation program for IBM and compatible personal computers. *In* RH Loeppert, AP Schwab, S Goldberg, eds, Chemical Equilibrium and Reaction Models (special publication no. 2). Soil Science Society of America, Madison, WI, pp 253–269

Pence NS, Larsen PB, Ebbs SD, Letham DLD, Lasat MM, Garvin DF, Eide D, Kochian LV (2000) The molecular physiology of metal transport in the Zn/Cd hyperaccumulator, *Thlaspi caerulescens*. Proc Natl Acad Sci USA **97:** 4956–4960

Reid RJ, Brookes JD, Tester MA, Smith FA (1996). The mechanism of Zn uptake in plants: characterization of low affinity system. Planta **198:** 39–45

Rengel Z (1999) Physiological mechanisms underlying differential nutrient efficiency of crop genotypes. *In* Z Rengel, ed, Mineral Nutrition of Crops. Food Products Press, New York, pp 231–269

Rengel Z, Graham RD (1995) Wheat genotypes differ in Zn efficiency when grown in chelate-buffered nutrient solution: II. Nutrient uptake. Plant Soil **176:** 317–324

Rengel Z, Wheal MS (1997). Kinetic parameters of Zn uptake by wheat are affected by the herbicide chlorsulfuron. J Exp Bot **48:** 935–941

Sillanpaa M (1982) Micronutrients and the nutrient status of soils: a global study. FAO Soils Bull **48:** 75–82

Swietlik D (1989) Zinc stress on citrus. J Rio G Valley Hort Sci **42:** 87–95

von Wiren N, Marschner H, Romheld V (1996) Roots of iron-efficient maize also absorb phytosiderophore-chelated zinc. Plant Physiol **111:** 1119–1125

Welch RM (1995) Micronutrient nutrition of plants. Crit Rev Plant Sci **14:** 49–87

Welch RM, Webb MJ, Loneragan JF (1982) Zinc in membrane function and its role in P toxicity. *In* A Scaife, ed, Plant Nutrition, 1982 Proceedings of the Ninth Plant Nutrition Colloquium Commonwealth Agricultural Bureau, Warwick, UK, pp 710–715

Wheal M, Rengel Z (1997) Chlorsulfuron reduces rates of zinc uptake by wheat seedlings from solution culture. Plant Soil **188:** 309–317