

*Full Length Research Paper*

# Does zinc uptake relate well with differential zinc efficiency of barley genotypes?

Emin Bülent ERENOĞLU

Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Çukurova University, Balcalı, Sarıçam 01330 Adana, Turkey.

Received 21 August, 2018; Accepted 18 September, 2018

To some extent, differential zinc (Zn) efficiency of cereals, particularly differences between species may be attributed to phytosiderophores (PSs) release and inorganic Zn ( $Zn^{2+}$ ) uptake; however, the discrepancies within a given species are still under discussion. Moreover, studies on the explanations of differential Zn efficiencies of barleys are limited. That is why using two barley (*Hordeum vulgare* L.) cultivars (*Zn-efficient*, Tarm-92 and *-inefficient*, Hamidiye-79), two short-term uptake experiments were designed with two forms of Zn, free Zn ( $Zn^{2+}$ ) or PS chelated Zn (Zn-PS) labelled with radioactive Zn ( $^{65}Zn$ ) in nutrient solution culture. Similar to earlier studies, the Zn uptake by roots and its translocation to shoots of barley supplied as either free ( $Zn^{2+}$ ) or chelated (Zn-PS) was induced under Zn deficiency. Although according to results of previous works, a close relationship between  $Zn^{2+}$  uptake of roots and Zn efficiencies of the same barley cultivars might have existed, the outcomes of the present research showed the opposite. Neither the uptake of  $Zn^{2+}$  and Zn-PS from roots nor their translocation to shoot had any compatible connection with the Zn efficiencies of barley cultivars. So the reason for differential Zn efficiency within a given cereal species remained unclear including barley as well.

**Key words:** Barley, phytosiderophores, uptake, zinc, zinc efficiency.

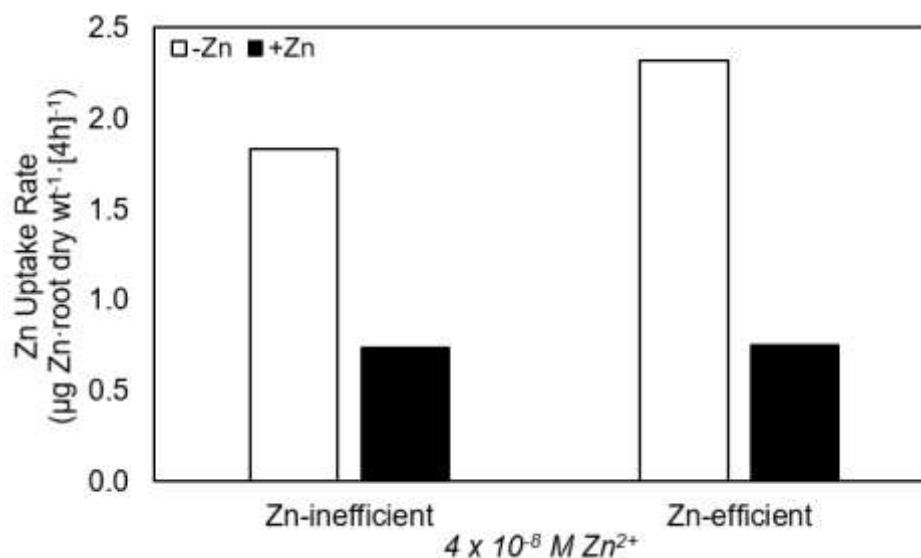
## INTRODUCTION

Zinc (Zn) deficiency is one of the well-recognized micronutrient deficiencies all over the world and particularly in calcareous soils of arid and semi-arid regions (Cakmak et al., 1996a). The use of Zn containing fertilizers to eliminate the problem of Zn deficiency is a typical application. However, plant species (Moraghan, 1984) and cultivars within a species, mainly wheat (Cakmak et al., 1996b, 1998; Graham et al., 1992) significantly differ in their ability to take up Zn from soils

or to utilize this absorbed Zn internally. In the light of such genotypical differences, the importance of breeding genotypes with higher efficiency in Zn uptake from soils or utilization of Zn in plants increases. Also, the genotypes of a given species show substantial differences in sensitivity to Zn deficiency. As in wheat and barley, there is a significant genotypic variation in Zn efficiency (Graham et al., 1992). The barley cultivars, Tarm-92 (Zn-efficient) and Hamidiye-79 (Zn-efficient)

E-mail: berenoglu@cukurova.edu.tr. Tel: + 90 533 301 3213.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)



**Figure 1.** Inorganic Zn uptake rates of *Zn-inefficient* (cv. Hamidiye-79) and *-efficient* (cv. Tarm-92) barley cultivars used in present work (adapted from Erenoglu et al., 1997).

used in the present study differ in their sensitivity to Zn deficiency in the field (Yilmaz et al., 1996) and greenhouse conditions (Cakmak et al., 1998; Sadeghzadeh et al., 2016).

Even though extensive studies have been conducted, particularly for wheat genotypes, the reason for differential Zn efficiency of cereals is still not well understood. For example, differences in root morphology (Dong et al., 1995), release of Zn-mobilizing phytosiderophores (PSs) (Erenoglu et al., 1996) and Zn uptake capacity of roots (Cakmak et al., 1998) were discussed as possible responsible mechanisms for expression of Zn efficiency. Although so many research papers have been published concerning possible physiological mechanisms that are playing roles in differential efficiencies of wheat cultivars under Zn deficiency, studies with barley are limited. The release of Zn-mobilizing PSs (Erenoglu et al., 2000), Zn<sup>2+</sup> uptake (Erenoglu et al., 1997), and root exudation (Rasouli-Sadaghiani et al., 2011) are examples for those limited studies.

Graminaceous species increase the synthesis and release of non-protein amino acids, called PSs to the rhizosphere, under deficiencies of Fe (Römheld, 1987; Takagi, 1976) or Zn (Erenoglu et al., 1996, 2000; Hopkins et al., 1997; Zhang et al., 1989). It was also the case for barley that the Zn deficient plants released PSs but not as much as Fe deficient ones (Erenoglu et al., 2000). The well-known higher sensitivity of durum wheat to Zn deficiency (Rengel and Graham, 1995; Cakmak et al., 1996a) was ascribed to their lower PS release rates from roots (Erenoglu et al., 1996). However, the observation of such close relationship was always not possible, as it

happened with bread cultivars having different Zn efficiency. Erenoglu et al. (1996) found out that the genotypic differences in Zn efficiency among the bread wheat genotypes were not well related to the rate of PS release. In the case of barley cultivars, Erenoglu et al. (2000) showed that the Zn-efficient barley cultivar Tarm-92 had released higher amounts of PSs than the Zn-inefficient Hamidiye-79.

In long-term experiments under field conditions (Yilmaz et al., 1996) as well as under greenhouse conditions (Cakmak et al., 1998; Genc et al., 2004), Zn-efficient barley genotypes had a higher Zn uptake capacity than Zn-inefficient ones. In a short-term experiment conducted using chelator-buffered nutrient solution culture under controlled environmental conditions, Zn-efficient barley also had a greater Zn<sup>2+</sup> uptake rate than a Zn-inefficient one (Figure 1) (Erenoglu et al., 1997). However, up to date, ZnPS uptake abilities of barley cultivars differing in Zn efficiency were not compared in a scientific research paper.

Under the light of what is described above two short-term uptake experiments were conducted to see the roles of different Zn forms -Zn<sup>2+</sup> and ZnPS- in differential Zn efficiencies of barley cultivars using plants pre-cultured with or without Zn supply in nutrient solution culture in a climate chamber under controlled environmental conditions. In the first experiment, the Zn-efficient and -inefficient cultivars were compared for disclosure of the relationship between their Zn efficiencies and Zn<sup>2+</sup> and ZnPS uptake capacities at 1 x 10<sup>-6</sup> M concentrations of both Zn forms. Erenoglu et al. (1997) had already observed a close relationship between Zn efficiencies of both cultivars and their Zn<sup>2+</sup> uptake capacities in a

HEDTA chelator-buffered nutrient solution with  $4 \times 10^{-8}$  M free  $Zn^{2+}$  activity. That is why the second experiment was planned only with ZnPS to realize if the ZnPS uptake of both cultivars would differ by their Zn efficiency levels at a lower ZnPS concentration ( $4 \times 10^{-8}$  M).

## MATERIALS AND METHODS

### Plant materials and pre-culture

Two barley (*Hordeum vulgare* L. cvs. Tarm-92 and Hamidiye-92) cultivars were used in two independent nutrient solution experiments under controlled environmental conditions (a light/dark regime of 16/8 h, 24/20°C, 65-75% relative humidity, and a photosynthetic photon flux density of  $300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  at plant height provided by Sylvania FR 96 T lamps). Both cultivars had different Zn efficiency scores; and according to their performances in a field (Yilmaz et al., 1996) and a greenhouse (Cakmak et al., 1998) experiments, Tarm-92 and Hamidiye-92 were classified as Zn-efficient and -inefficient, respectively.

For both experiments, surface-sterilized seeds of barley cultivars were germinated in quartz sand moistened with saturated  $\text{CaSO}_4$  solution. After 5 days, seedlings were transferred to 2.8-L plastic pots (20 seedlings per pot) containing continuously aerated nutrient solution prepared as follows: (in mM): 0.88  $\text{K}_2\text{SO}_4$ , 2.0  $\text{Ca}(\text{NO}_3)_2$ , 0.25  $\text{KH}_2\text{PO}_4$ , 1.0  $\text{MgSO}_4$ , 0.1 KCl, and as  $\mu\text{M}$  100 Fe(III)EDTA, 1  $\text{H}_3\text{BO}_3$ , 0.5  $\text{MnSO}_4$ , 0.2  $\text{CuSO}_4$ , and 0.02  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ . The Zn was supplied in the form of  $\text{ZnSO}_4$  at concentrations of 0  $\mu\text{M}$  (for -Zn plants) and 1  $\mu\text{M}$  (for +Zn plants). For both experiments, plants were pre-cultured in nutrient solution for 11 days; and to have a nutrient-free apoplast during short-term uptake experiment, they were removed into a micronutrient free solution for one day.

### $Zn^{2+}$ uptake vs. ZnPS uptake (Experiment I)

On day 12, a part of the plants was transferred into 0.5-L containers (each contained 2-bundles with two plants) and supplied with  $1 \times 10^{-6}$  M  $\text{ZnSO}_4$  or ZnPSs (hydroxymugineic -acid) + 500% excess of PSs, labeled with  $^{65}\text{Zn}$  (46 KBq) for 3 h in freshly prepared micronutrients free nutrient solution. Four replicas were used to represent each treatment. After the uptake period, the roots were washed with 1 mM  $\text{CaSO}_4$  and 1 mM NaEDTA for 10 min to remove extracellular  $^{65}\text{Zn}$ , respectively.

Following the termination of the experiment, each bundle in each pot was collected as one replica, and in total, each treatment had four independent replicates. The roots and shoots were harvested, dried, and ashed at 550°C. After that, the ashed samples were dissolved in HCl and assayed for  $^{65}\text{Zn}$  activity by liquid scintillation spectrometry.

For determining Zn nutritional status of plants grown for 12 days with or without Zn supply, the plants were harvested, separated into shoot and roots, oven dried (70°C), ashed at 500°C, dissolved in 1% HCl, and later on, analyzed using AAS (Atomic Absorption Spectroscopy). Four replicas were examined to represent each treatment.

### Zinc uptake at low ZnPS supply (Experiment II)

After 11 days of pre-culture, the plants were transferred to micronutrients free solution and on day 12, a part of them was moved into 5-L containers (each contained 2-bundles with two plants) and supplied with  $4 \times 10^{-8}$  M ZnPSs (hydroxymugineic acid) + 500% excess of PSs, labeled with  $^{65}\text{Zn}$  (185 kBq) for 8 h in

freshly prepared micronutrients free nutrient solution. In this experiment as well, four replicas were used to represent each treatment. Root washing after uptake period; preparation of samples; and measuring the activities in them were also done as explained in Experiment I.

## Statistics

Experiments were designed using four replicas for each treatment, and the results of each trait were analyzed using ANOVA and Duncan's test at  $p < 0.05$ .

## RESULTS

### Experiment I

For clarifying the relationships between the Zn efficiencies of two barley cultivars and their Zn uptake capacities, a short-term uptake experiment was set up. Earlier results obtained using both varieties emphasized that there were relations between Zn efficiencies of both cultivars and their phytosiderophore releases (Erenoglu et al., 2000) and  $Zn^{2+}$  uptake (Erenoglu et al., 1997). In such a way, that the Zn-efficient cv. Tarm-92 reached up to higher PSs release rates and absorbed higher  $Zn^{2+}$  in comparison to the Zn-inefficient cv. Hamidiye-79.

After 12 days of preculture with or without Zn application, some growth parameters such as shoot and root dry weights and roots to shoot ratios (Table 1) and Zn contents per shoot or root dry weights (Table 2) of both cultivars revealed that the Zn-deficient plants of both varieties were suffering from Zn deficiency. Although the root dry weights of both cultivars were not affected by Zn composition of the nutrient solution, the shoot growths were significantly regressed by the non-sufficient supply of Zn. Because of this, roots to shoot ratios of plants grown without Zn were higher than control plants. Following these results, when the plants were supplied with deficient Zn, the Zn concentrations in shoot and roots of both cultivars decreased very drastically (Table 2).

Both barley cultivars pre-cultured without Zn tended to take more  $Zn^{2+}$  and ZnPS up compared to those pre-cultured with Zn supply (Figure 2a). However, Zn deficiency induced Zn uptake was much more evident for  $Zn^{2+}$  than Zn-PS. While the Zn-deficient plants absorbed up to 5.1-fold (Zn-efficient cv.) more  $Zn^{2+}$  in comparison to Zn-sufficient plants, the increment for Zn-phytosiderophores uptake was only 1.5-fold (Zn-efficient cv.). For neither inorganic Zn nor Zn-PS, there were no distinct differences among the cultivars. In fact, the Zn efficient cultivar, particularly in the case of Zn-PS, showed lower Zn uptake capacity than the Zn inefficient one.

When it comes to transport of absorbed Zn from roots to shoot, Figure 2b shows a similar tendency to that of the root uptake values. Both barley cultivars pre-cultured

**Table 1.** Shoot and roots dry weights and roots/shoot ratios of barley cultivars with different Zn efficiency. The plants were pre-cultured with or without Zn application for 11 days after five days germination period; and on day 17, supplied with  $1 \times 10^{-6}$  M  $^{65}\text{Zn}^{2+}$  (as  $\text{ZnSO}_4$ ) or  $^{65}\text{Zn}$ -PS (chelated with hydroxymugineic acid) for 3 h.

Parameter	Dry weights (mg plant <sup>-1</sup> )					
	Shoot		Roots		Roots/Shoot	
	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn
<b>1 x 10<sup>-6</sup> M Zn</b>						
Zn-inefficient	47 <sup>d</sup>	64 <sup>abc</sup>	20 <sup>c</sup>	18 <sup>c</sup>	0.42 <sup>a</sup>	0.27 <sup>b</sup>
Zn-efficient	62 <sup>bc</sup>	74 <sup>ab</sup>	26 <sup>abc</sup>	24 <sup>abc</sup>	0.42 <sup>a</sup>	0.32 <sup>b</sup>
<b>1 x 10<sup>-6</sup> M ZnPS</b>						
Zn-inefficient	47 <sup>d</sup>	60 <sup>dc</sup>	18 <sup>c</sup>	17 <sup>c</sup>	0.39 <sup>a</sup>	0.29 <sup>b</sup>
Zn-efficient	60 <sup>dc</sup>	78 <sup>a</sup>	25 <sup>ab</sup>	25 <sup>ab</sup>	0.42 <sup>a</sup>	0.32 <sup>b</sup>

Values are the means of four independent replicates. For each trait, numbers with different letters are significantly different from each other at  $p < 0.05$  according to ANOVA and Duncan's test.

**Table 2.** Zinc concentrations in shoot and roots of barley cultivars with different Zn efficiency. The plants were pre-cultured with or without Zn application for 11 days after five days germination period.

Parameter	Zn concentration ( $\mu\text{g g}^{-1}$ )			
	Shoot		Roots	
	-Zn	+Zn	-Zn	+Zn
Zn-inefficient	5.7 <sup>c</sup>	56 <sup>a</sup>	9.3 <sup>c</sup>	71 <sup>b</sup>
Zn-efficient	4.3 <sup>c</sup>	49 <sup>b</sup>	9.0 <sup>c</sup>	87 <sup>a</sup>

Values are the means of four independent replicates. For each trait, numbers with different letters are significantly different from each other at  $p < 0.05$  according to ANOVA and Duncan's test

without Zn tended to translocate more  $\text{Zn}^{2+}$  and Zn-PS from roots to shoot in comparison to those pre-cultured with Zn supply (Figure 2b). Moreover, the effect of Zn deficiency on the induction of this translocation was almost twice higher in comparison to the induction of uptake. Opposite to very distinct differences in Zn deficiency induced Zn uptake values for  $\text{Zn}^{2+}$  than Zn-PS, the distinctions in Zn translocation values for  $\text{Zn}^{2+}$  than Zn-PS became less. It is to say that; while the Zn-deficient plants translocated up to 9.6-fold (Zn-efficient cv.) more  $\text{Zn}^{2+}$  in comparison to Zn-sufficient plants, the increment for Zn-PS uptake was 3.8-fold (Zn-efficient cv.). Among the cultivars, there were no apparent differences for neither inorganic Zn nor Zn-PS translocations.

## Experiment II

As mentioned above, in an earlier study conducted using chelator-buffered nutrient solution, a clear relationship between Zn efficiencies of both cultivars and their  $\text{Zn}^{2+}$  uptake at  $4 \times 10^{-8}$  M free  $\text{Zn}^{2+}$  activity had already been

shown (Erenoglu et al., 1997). That is why another short-term uptake experiment was set up to study the relationships between Zn efficiencies of these two barley cultivars and their Zn uptake from a solution with  $4 \times 10^{-8}$  M final Zn-PS concentration.

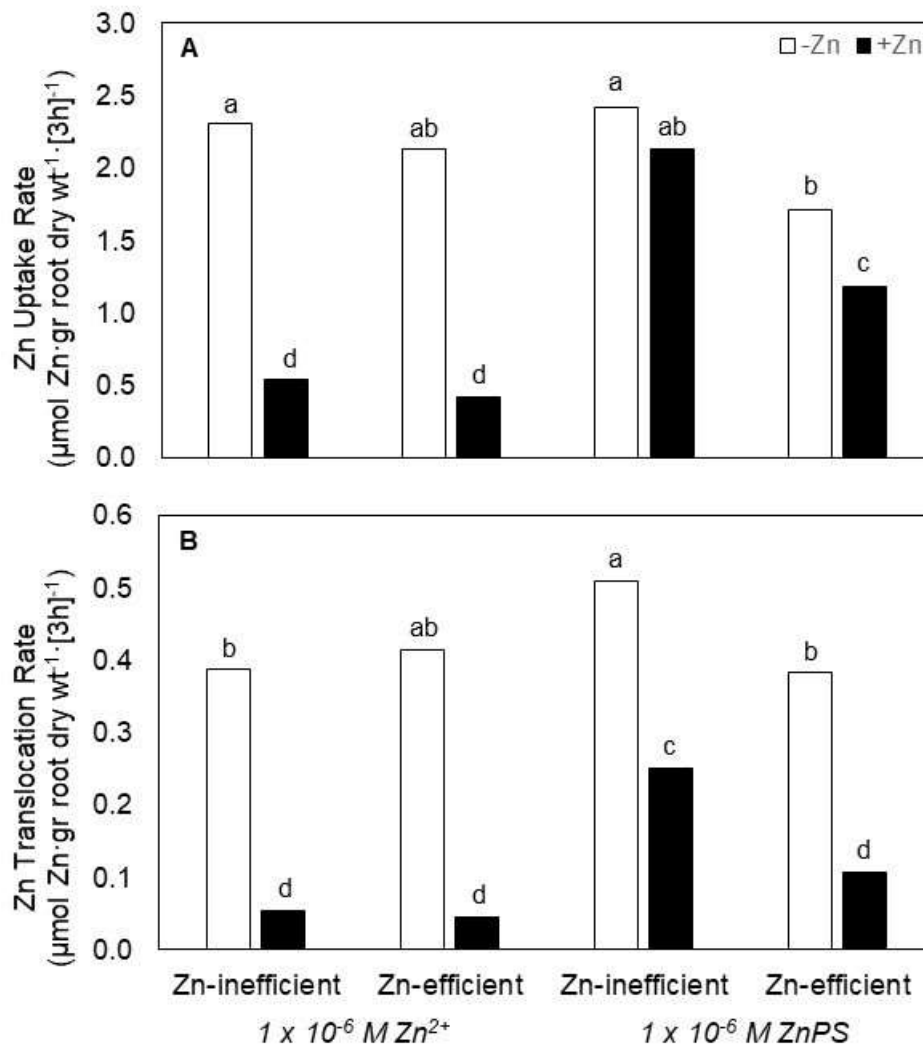
After 12 days of pre-culture with or without Zn application, some growth parameters such as shoot and root dry weights and roots to shoot ratios of both cultivars harvested after the experiment revealed that the Zn-deficient plants of both cultivars were in Zn deficiency stress (Table 3). Since the behaviours of both varieties were similar to their performances in Experiment I, these observations are not given here.

Parallel to the results obtained in Experiment I, both barley cultivars pre-cultured without Zn showed a tendency to take more Zn-PS up compared to those pre-cultured with Zn supply (Figure 3a). However, although 25 times lower Zn-PS concentration than Experiment I was applied, similar to Experiment I, no apparent differences in Zn absorption of both cultivars were found. Moreover, the induction of Zn-PS uptake under Zn deficiency for both varieties was in similar range given for Experiment I.

Roots to shoot transport of Zn-PS in both cultivars are presented in Figure 3b. Similar to Experiment I, although there was no apparent genotypical difference, the amounts of Zn-PS translocated from roots to shoot increased in both varieties.

## DISCUSSION

As it is in well-agreement with earlier studies (Erenoglu et al., 1996, 2000), the growth parameters and tissue Zn concentrations in shoot and roots of barley cultivars (Tables 1 and 2) were negatively affected from the deficient supply of Zn. It is to say that the Zn-deficient plants showed declined shoot growth, enhanced roots-to-shoot ratios, and dramatically decreases in Zn

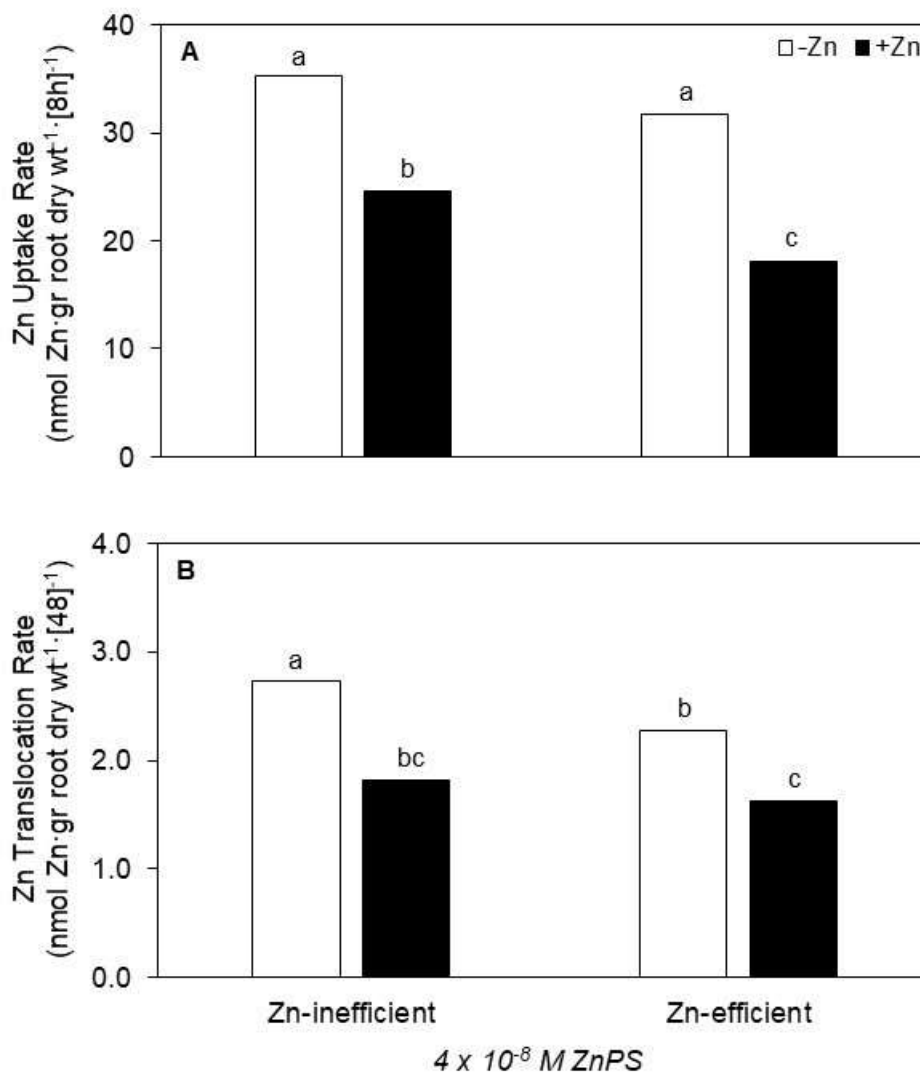


**Figure 2.** Zinc uptake and root-to-shoot translocation rates of barley cultivars with different Zn efficiency. The plants were pre-cultured with or without Zn application for 11 days after five days germination period; and on day 17, supplied with  $1 \times 10^{-6} M$   $^{65}Zn^{2+}$  (as  $ZnSO_4$ ) or  $^{65}Zn$ -PS (chelated with hydroxymugineic acid) for 3 h. Values are the means of four independent replicates. For each trait, numbers with different letters are significantly different from each other at  $p < 0.05$  according to ANOVA and Duncan's test.

**Table 3.** Shoot and roots dry weights and roots/shoot ratios of barley cultivars with different Zn efficiency. The plants were pre-cultured with or without Zn application for 11 days after five days germination period; and on day 17, supplied with  $4 \times 10^{-8} M$   $^{65}Zn$ -PS (chelated with hydroxymugineic acid) for 8 hours.

Parameter	Dry weights (mg plant <sup>-1</sup> )				Roots/Shoot	
	Shoot		Roots		-Zn	+Zn
	-Zn	+Zn	-Zn	+Zn		
$4 \times 10^{-8} M ZnPS$						
Zn-inefficient	65 <sup>b</sup>	86 <sup>a</sup>	45 <sup>ab</sup>	34 <sup>b</sup>	0.69 <sup>a</sup>	0.40 <sup>c</sup>
Zn-efficient	81 <sup>a</sup>	95 <sup>a</sup>	49 <sup>a</sup>	41 <sup>a</sup>	0.61 <sup>b</sup>	0.43 <sup>c</sup>

Values are the means of four independent replicates. For each trait, numbers with different letters are significantly different from each other at  $p < 0.05$  according to ANOVA and Duncan's test.



**Figure 3.** Zinc uptake (A) and root-to-shoot translocation (B) rates of barley cultivars with different Zn efficiency. The plants were pre-cultured with or without Zn application for 11 days after five days germination period; and on day 17, supplied with  $4 \times 10^{-8} \text{ M } ^{65}\text{Zn-PS}$  (chelated with hydroxymugineic acid) for 8 h. Values are the means of four independent replicates. For each trait, numbers with different letters are significantly different from each other at  $p < 0.05$  according to ANOVA and Duncan's test.

concentrations of the shoot and root tissues.

As scientifically well proven, plant species (Moraghan, 1984) and cultivars within a species, particularly in wheat (Cakmak et al., 1996b, 1998; Graham et al., 1992) significantly differ in their ability to take Zn up from soils or to utilize it internally. As in wheat, there are also sizeable genotypic differences among the barley cultivars concerning Zn efficiency (Graham et al., 1992; Yilmaz et al., 1996; Cakmak et al., 1998; Sadeghzadeh et al., 2016).

A large number of mechanisms contributing to Zn efficiency have been proposed which might be operative either in the rhizosphere or within plants; for example,

differences in root morphology, mycorrhizal infection, the release of Zn-mobilizing PSs, uptake, translocation, and compartmentation of Zn (Graham and Rengel, 1993). Shortly after this introduction, the reason for differential Zn efficiency of wheat genotypes was extensively studied but is still not well understood. Enhanced root growth (Dong et al., 1995), release of Zn-mobilizing PSs from roots (Cakmak et al., 1998), and an increased Zn uptake capacity of roots (Cakmak et al., 1998) were suggested as possible parameters determining Zn efficiency. For barley, only the release of PSs was investigated in detail among these possible mechanisms in differential Zn efficiencies of a species or cultivars within a species

(Erenoglu et al., 2000). Besides, the potential role of  $Zn^{2+}$  uptake in Zn efficiency of the same barley cultivars was evaluated in short-term uptake experiment set up in a chelator-buffered nutrient solution (Erenoglu et al., 1997).

In accordance with an earlier study conducted using barley cultivars in chelator-buffered nutrient solution (Erenoglu et al., 1997), the Zn uptake by roots of barley plants is induced under Zn deficiency in conventional nutrient solution (Figures 2 and 3). Besides, this output is similar to those found in other cereals as well (Cakmak et al., 1998; Rengel and Hawkesford, 1997; Rengel and Wheal, 1997). Possibly, this induction in  $Zn^{2+}$  uptake is due to one (or combination) of the six members of ZIP family transporters which were recently found in barley suffering from Zn deficiency (Tiong et al., 2015).

As it was clearly proved, to some extent, differential Zn efficiency of cereals may be attributed to  $Zn^{2+}$  uptake capacities of cereals, particularly when compared to bread wheat, higher Zn efficiency of rye and lower efficiency of durum wheat to their higher and lower  $Zn^{2+}$  uptake abilities, respectively (Cakmak et al. 1998). However, up till now, the reason for a higher Zn uptake rate of rye or lower Zn uptake of durum wheat under deficient supply of Zn is not scientifically clarified yet. Differences in the Zn uptake rates are also known within genotypes of a given cereal species such as sorghum (Ramani and Kannan, 1985), bread wheat (Rengel and Wheal, 1997), and barley (Erenoglu et al., 1997). However, no clear difference could be found between Zn-efficient and Zn-inefficient bread wheat cultivars in either uptake or root-to-shoot translocation rates of Zn (Cakmak et al. 1998). In the present study, the Zn-efficient and -inefficient barley cultivars did not show any consistency between their efficiencies and  $Zn^{2+}$  uptake (Figure 2a). This result was surprising since the  $Zn^{2+}$  absorptions of same cultivars had reflected perfect accordance with their Zn efficiencies in a chelator-buffered nutrient solution supplied with  $4 \times 10^{-8}$  M free Zn in previous work (Erenoglu et al., 1997). The reason for such discrepancy is not known and may be the result of different experimental conditions (that is, use of a chelate-buffered nutrient solution or 25 times lower free Zn activity than present experiment).

As it is well-known, graminaceous plant species increase capacities for PS release and Fe(III)-PS absorption under iron deficiency (Römheld and Marschner, 1986). In roots of maize high-affinity Fe(III)-phytosiderophore uptake is necessary to produce healthy plants and is strongly dependent on the YS1 gene (von Wiren et al., 1994) and there is the stoichiometric uptake of metal and ligand (von Wiren et al., 1995). Besides Fe, while the putative Fe-PSs transporter in maize (*Zea mays* L.) roots recognizes Zn-PS (von Wiren et al., 1996), ZmYS1 complements the growth defect of the zinc uptake-defective yeast mutant zap1 and transports PS-bound Zn into oocytes (Schaaf et al., 2004a). In parallel, the results of the present paper indicated that barley

could also take Zn-PS up (Figures 2a and 3a) and translocate it into shoots (Figures 2b and 3b). However, the opposite of  $Zn^{2+}$  uptake (Figure 2a), the inductive effect of Zn deficiency on Zn-PS uptake (Figures 2a and 3a) was very low. In agreement with this, in leaves of maize, while Fe deficiency upregulated ZmYS1 transcript levels very strongly, Zn deficiency had a minimal effect on it (Schaaf et al., 2004b). As it is for  $Zn^{2+}$  uptake (Figure 2a) and translocation (Figure 2b), no apparent relation exists between Zn efficiencies and Zn-PS uptake (Figures 2a and 3a) or its translocation into shoots (Figures 2b and 3b).

As mentioned above, in a previous study conducted using the same barley cultivars, a positive relationship between Zn efficiencies and  $Zn^{2+}$  uptake rates had been observed (Erenoglu et al., 1997); however, in the present study, this relation disappeared (Figure 2a). Besides, lower free Zn activity in chelate-buffered nutrient solution was mentioned as one of the possible reasons for this discordance. Nevertheless, the barley cultivars having differential Zn efficiencies did not show any differences concerning their Zn efficiencies for either ZnPS uptake or translocation (Figures 3a and 3b) even at a lower Zn concentration ( $4 \times 10^{-8}$  M) compared to the mentioned study (Erenoglu et al. 1997).

## Conclusion

In line with earlier studies (Cakmak et al., 1998; Erenoglu et al., 1997, 1999; Rengel and Hawkesford, 1997; Rengel and Wheal, 1997), the Zn uptake by roots of barley supplied as either free ( $Zn^{2+}$ ) or chelated (Zn-PS) was induced under Zn deficiency (Figures 2a and 3a). However, the induction was much apparent for  $Zn^{2+}$  than Zn-PS. Although according to results of previous works (Erenoglu et al., 1997) that a close relationship between  $Zn^{2+}$  uptake capacity of roots and Zn efficiencies of the same barley cultivars might have existed, the outcomes of present research paper showed the opposite. In such a way, that neither the uptake of  $Zn^{2+}$  and Zn-PS from roots (Figures 2a and 3a) nor their translocation from roots-to-shoot (Figures 2b and 3b) had no compatible connection to the Zn efficiencies of barley cultivars. So the reason(s) for differential Zn efficiency within a given cereal species remained unclear including barley. Internal utilization efficiency might also be considered as a possible mechanism in differential efficiencies in cultivars of a species. Also, such differences within a species might also be combined results of multiple mechanisms, which are not easily followed experimentally in laboratory conditions.

## ACKNOWLEDGEMENTS

The author thank DFG-Deutsche Forschungsgemeinschaft for the financial support and

Prof. Volker Römheld (†) for his guidance during the lab-works. This manuscript was prepared by using a part of the results obtained from the research project funded by DFG-Deutsche Forschungsgemeinschaft (Project No. DFG-RO 796/10).

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

## REFERENCES

- Cakmak I, Gülüt KY, Marschner H, Graham RD (1994). Effect of zinc and iron deficiency on phytosiderophore release in wheat genotypes differing in zinc efficiency. *Journal of Plant Nutrition* 17:1-17.
- Cakmak I, Sari N, Marschner H, Kalayci M, Yilmaz A, Eker S, Gülüt KY (1996b). Dry matter production and distribution of zinc in bread wheat and durum wheat genotypes differing in zinc efficiency. *Plant and Soil* 180:173-181.
- Cakmak I, Torun B, Erenoglu B, Oztürk L, Marschner H, Kalayci M, Ekiz H, Yilmaz A. (1998). Morphological and physiological differences in the response of cereals to zinc deficiency. *Euphytica* 100:349-357.
- Cakmak I, Yilmaz A, Kalayci M, Ekiz H, Torun B, Erenoglu B, Braun HJ (1996a). Zinc deficiency as a critical problem in wheat production in Central Anatolia. *Plant and Soil* 180:165-172.
- Dong B, Rengel Z, Graham RD (1995). Root morphology of wheat genotypes differing in zinc efficiency. *Journal of Plant Nutrition* 18:2761-2773.
- Erenoglu B, Cakmak I, Römheld V, Derici R, Rengel Z (1999). Uptake of zinc by rye, bread wheat, and durum wheat cultivars differing in zinc efficiency. *Plant and Soil* 209:245-252.
- Erenoglu B, Cakmak I, Marschner H, Römheld V, Eker S, Daghan H, Kalayci M, Ekiz H (1996). Phytosiderophore release does not relate well with Zn efficiency in different bread wheat genotypes. *Journal of Plant Nutrition* 19:1569-1580.
- Erenoglu B, Eker S, Cakmak I, Derici R, Römheld V (1997). Effect of Zn deficiency on zinc (65Zn) uptake of some barley cultivars (in Turkish). In: I. National Zinc Congress, 12-16 May 1997. Eskisehir, Turkey.
- Erenoglu B, Eker S, Cakmak I, Derici R, Römheld V (2000). Effect of iron and zinc deficiency on release of phytosiderophores in barley cultivars differing in zinc efficiency. *Journal of Plant Nutrition* 23:1645-1656.
- Genc Y, McDonald GK, Graham RD (2004). Differential expression of zinc efficiency during the growing season of barley. *Plant and Soil* 263:273-282.
- Graham RD, Aschner JS, Hynes SC (1992). Selecting zinc-efficient cereal genotypes for soils of low zinc status. *Plant and Soil* 146:241-250.
- Graham RD, Rengel Z (1993). Genotypic variation in zinc uptake and utilization by plants. In *Zinc in Soils and Plants*. Ed. A D Robson. pp. 107-118. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Hopkins BG, Whitney DA, Lamond RE, Jolley VD (1997). Phytosiderophore release by sorghum, wheat, and corn under zinc deficiency. *Journal of Plant Nutrition* 21:2623-2637.
- Moraghan JT (1984). Differential response of five species to phosphorus and zinc fertilizers. *Communications in Soil Science and Plant Analysis* 15:437-447.
- Ramani S, Kannan S (1985). Zinc absorption and transport in young peanut seedlings. *Communications in Soil Science and Plant Analysis* 9:311-316.
- Rasouli-Sadaghiani MH, Sadeghzadeh B, Sepehr E, Rengel Z (2011). Root exudation and zinc uptake by barley genotypes differing in Zn efficiency. *Journal of Plant Nutrition* 34:1120-1132.
- Rengel Z, Graham RD (1995). Wheat genotypes differ in zinc efficiency when grown in the chelate-buffered nutrient solution. I. Growth. *Plant and Soil* 176:307-316.
- Rengel Z, Hawkesford MJ (1997). Biosynthesis of a 34-kDa polypeptide in the root-cell plasma membrane of a Zn-efficient wheat genotype increases upon Zn deficiency. *Australian Journal of Plant Physiology* 24: 307-315.
- Rengel Z, Wheal WS (1997). Kinetic parameters of zinc uptake by wheat are affected by the herbicide chlorsulfuron. *Journal of Experimental Botany* 48:935-941.
- Römheld V (1987). Different strategies for iron acquisition in higher plants. *Physiologia Plantarum* 70:231-234.
- Römheld V, Marschner H (1986). Evidence for a specific uptake system for iron phytosiderophore in roots of grasses. *Plant Physiology* 80:175-180.
- Sadeghzadeh B, Sadeghzadeh N, Sepehr E (2016). Barley Genotypes Differing in Zinc Efficiency When Grown in Various Soil Types. *International Journal of Plant and Soil Science* 12(4):1-13.
- Schaaf G, Erenoglu BE, von Wirén N (2004a). Physiological and biochemical characterization of metal-phytosiderophore transport in graminaceous species. *Soil Science and Plant Nutrition* 50: 989-995.
- Schaaf G, Ludewig U, Erenoglu BE, Mori S, Kitahara T, von Wirén N (2004b). ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. *Journal of Biological Chemistry* 279:9091-9096.
- Takagi S (1976). Naturally occurring iron-chelating compounds in oat and rice-root washings. I. Activity measurements and preliminary characterization. *Soil Science and Plant Nutrition* 22:423-433.
- Tiong J, McDonald G, Genc Y, Shirley N, Langridge P, Huang CY (2015). Increased expression of six ZIP family genes by zinc (Zn) deficiency is associated with enhanced uptake and root-to-shoot translocation of Zn in barley (*Hordeum vulgare*). *New Phytologist* 207:1097-1109
- Von Wirén N, Marschner H, Römheld V (1995). Uptake kinetics of iron-phytosiderophores in two maize genotypes differing in iron efficiency. *Physiologia Plantarum* 93:611-616.
- Von Wirén N, Marschner H, Römheld V (1996). Roots of iron inefficient maize (*Zea mays* L.) take up also phytosiderophore chelated zinc. *Plant Physiology* 111:1119-1125.
- Von Wirén N, Mori S, Marschner H, Römheld V (1994). Iron inefficiency in maize mutant *ysl* (*Zea mays* L. cv Yellow-Stripe) is caused by a defect in uptake of iron phytosiderophores. *Plant Physiology* 106:71-77.
- Yilmaz A, Kalayci M, Ekiz H, Torun B, Oztürk L, Cakmak I (1996). Response of different barley genotypes to zinc fertilization in Central Anatolia (in Turkish). In: III. Malt and Barley Symposium, 5-7 September 1995. Konya, Turkey.
- Zhang F, Römheld V, Marschner H (1989). Effect of zinc deficiency in wheat on the release of zinc and iron mobilizing exudates. *Journal of Plant Nutrition and Soil Sciences* 152:205-210.