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Phytosiderophore release in *Aegilops tauschii* and *Triticum* species under zinc and iron deficiencies

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Abstract

Using three diploid (*Triticum monococcum*, AA), three tetraploid (*Triticum turgidum*, BBAA), two hexaploid (*Triticum aestivum* and *Triticum compactum*, BBAADD) wheats and two *Aegilops tauschii* (DD) genotypes, experiments were carried out under controlled environmental conditions in nutrient solution (i) to study the relationships between the rates of phytosiderophore (PS) release from the roots and the tolerance of diploid, tetraploid, and hexaploid wheats and *Ae. tauschii* to zinc (Zn) and iron (Fe) deficiencies, and (ii) to assess the role of different genomes in PS release from roots under different regimes of Zn and Fe supply. Phytosiderophores released from roots were determined both by measurement of Cu mobilized from a Cu-loaded resin and identification by using HPLC analysis. Compared to tetraploid wheats, diploid and hexaploid wheats were less affected by Zn deficiency as judged from the severity of leaf symptoms. *Aegilops tauschii* showed very slight Zn deficiency symptoms possibly due to its slower growth rate. Under Fe-deficient conditions, all wheat genotypes used were similarly chlorotic; however, development of chlorosis was first observed in tetraploid wheats. Correlation between PS release rate determined by Cu-mobilization test and HPLC analysis was highly significant. According to HPLC analysis, all genotypes of *Triticum* and *Ae. tauschii* species released only one PS, 2′-deoxymugineic acid, both under Fe and Zn deficiency. Under Zn deficiency, rates of PS release in tetraploid wheats averaged 1 μmol (30 plants)$^{-1}$ (3 h)$^{-1}$, while in hexaploid wheats rate of PS release was around 14 μmol (30 plants)$^{-1}$ (3 h)$^{-1}$. Diploid wheats and *Ae. tauschii* accessions behaved similarly in their capacity to release PS and intermediate between tetraploid and hexaploid wheats regarding the PS release capacity. All *Triticum* and *Aegilops* species released more PS under Fe than Zn deficiency, particularly when the rate of PS release was expressed per unit dry weight of roots. On average, the rates of PS release under Fe deficiency were 3.0, 5.7, 8.4, and 16 μmol (30 plants)$^{-1}$ (3 h)$^{-1}$ for *Ae. tauschii*, diploid, tetraploid and hexaploid wheats, respectively. The results of the present study show that the PS release mechanism in wheat is expressed effectively when three genomes, A, B and D, come together, indicating complementary action of the corresponding genes from A, B and D genomes to activate biosynthesis and release of PS.

Key words: *Aegilops tauschii*, iron deficiency, phytosiderophores, *Triticum monococcum*, *Triticum dicoccum*, *Triticum aestivum*, zinc deficiency.

Introduction

Zinc and Fe deficiencies are common micronutrient deficiencies in calcareous soils, and adversely affect crop production (Sillanpää, 1982; Vose, 1982; White and Zasoski, 1999). Zinc deficiency is a particular micronutrient deficiency problem in cereal-growing areas causing large decreases in grain yield and quality, for example in Australia (Graham *et al*., 1992), Turkey (Cakmak *et al*., 1996*a*, 1999*a*) and India (Takkar *et al*., 1989).

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There is substantial variation in tolerance to Zn or Fe deficiency within and among cereal species. Possibly, the release of phytosiderophores (PS) (non-protein amino acids) from roots in response to Fe or Zn deficiencies is an important factor affecting genotypic variation in the tolerance to Zn and Fe deficiencies. Phytosiderophores are highly effective in solubilization and mobilization of Zn and Fe in calcareous soils (Treeby *et al*., 1989) and are involved in the uptake of these nutrients by roots (Römheld and Marschner, 1990; von Wiren *et al*., 1995). The existence of large differences in tolerance to Fe deficiency between various cereal species correlated well with the release rate of PS from roots (Marschner *et al*., 1986; Kawai *et al*., 1988; Römheld and Marschner, 1990). Similarly, differences in tolerance to Zn deficiency between sorghum, wheat and corn correlate well with the amounts of PS released from roots (Hopkins *et al*., 1998). Wild grasses, adapted to severely Zn-deficient calcareous soils, released high amounts of PS when grown under Zn deficiency (Cakmak *et al*., 1996*c*). Bread wheat cultivars show greater tolerance to Zn deficiency than durum wheat cultivars, and this difference in tolerance correlated with differences in the release rate of phytosiderophores (Cakmak *et al*., 1994; Walter *et al*., 1994; Rengel *et al*., 1998). However, when genotypes of a given cereal species were compared, tolerance to Zn or Fe deficiencies and the rate of PS release were not always well correlated, as shown in oat for Fe deficiency (Hansen and Jolley, 1995) and wheat for Zn deficiency (Erenoglu *et al*., 1996).

Little information is available about the genetic control of PS release from roots. Since both the concentrations in roots and the amounts released from roots of PS are much lower in tetraploid (BBAA) than hexaploid wheats (BBAADD) under Fe and especially Zn deficiency (Cakmak *et al*., 1994, 1996*b*; Rengel and Romheld, 2000*a*), it can be assumed that the D genome possibly affects the biosynthesis and release of PS. Recently, it has been shown that diploid wheats (AA), like hexaploid wheats, possess very high tolerance to Zn deficiency when grown in Zn-deficient calcareous soils (Cakmak *et al*., 1999*b*). This may suggest a role of the A genome in the synthesis and release of PS. *Aegilops tauschii* (DD) is the donor of the D genome in hexaploid wheat (Kerby and Kuspira, 1987; Miller, 1987), and there is a high variation in tolerance to Zn deficiency between the accessions of *Ae. tauschii* (Cakmak *et al*., 1999*c*). Using only one genotype from each wheat species, it was recently shown (Ma *et al*., 1999) that diploid (AA), tetraploid (BBAA) and hexaploid wheats (BBAADD) and *Ae. tauschii* (DD) are able to release PS under Fe deficiency. In this study (Ma *et al*., 1999), the highest and lowest amounts of PS release were found in hexaploid and diploid wheats, respectively. In the present study, using three diploid (AA), three tetraploid (BBAA) and two hexaploid (BBAADD) wheats and two *Ae. tauschii* (DD) accessions experiments were carried out to study the role of different genomes on the release of PS under Fe and Zn deficiencies. Among the species studied, the diploid and hexaploid wheat genotypes are known to be tolerant to Zn deficiency, and tetraploid wheats and *Ae. tauschii* accessions show high and moderate sensitivity to Zn deficiency when grown on a severely Zn-deficient soil, respectively (Cakmak *et al*., 1999*b*, *c*).

Materials and methods

Plant growth

Two separate experiments were carried out to study the effects of genomes of the *Ae. tauschii* and *Triticum* species on PS release under Zn (experiment I) and Fe (experiment II) deficiencies. The genotypes of *Ae. tauschii* and *Triticum* species used in the present work are presented in Table 1. Seeds from Germany were provided by Dr CI Kling (University of Hohenheim-Stuttgart). Seeds, surface-sterilized by 1% (v/v) sodium hypochlorite for 20 min, were germinated in quartz sand moistened with saturated $CaSO_4$ solution. After 4 or 5 d the seedlings were transferred to 2.5 l plastic pots (30 seedlings per pot) containing the following continuously aerated nutrient solution: 0.88 mM K_2SO_4, 2.0 mM $Ca(NO_3)_2$, 0.25 mM KH_2PO_4, 1.0 mM $MgSO_4$,

Table 1. *Diploid, tetraploid and hexaploid wheats used in the study, and their genotypes and sources*

Species	Accessions or cultivars	Seed source	Classification
Diploid *T. monococcum* (AA)			
ssp. *monococcum*	FAL-67	Germany	Primitive
ssp. *monococcum*	FAL-43	Germany	Primitive
ssp. *monococcum*	FAL-30	Germany	Primitive
Tetraploid *T. turgidum* (BBAA)			
ssp. *dicoccum*	FAL-21	Germany	Primitive
ssp. *dicoccum*	FAL-02	Germany	Primitive
ssp. *dicoccum*	FAL-13	Germany	Primitive
Hexaploid *T. aestivum* (BBAADD)			
ssp. *compactum*	Stammbaum	Germany	Primitive
ssp. *aestivum*	Bezostaja	Turkey	Modern
Aegilops tauschii (DD)	400682	ICARDA	Wild
Aegilops tauschii (DD)	400630	ICARDA	Wild
Aegilops tauschii (DD)	400356	ICARDA	Wild

0.1 mM KCl, 1 μM H_3BO_4, 0.5 μM $MnSO_4$, and 0.02 μM $(NH_4)_6Mo_7O_{24}$. In the experiment dealing with Zn deficiency no Zn, but 0.1 mM Fe-EDTA was added, and in the experiment with Fe deficiency, plants were supplied with 1 μM Fe-EDTA and 1 μM $ZnSO_4$. Due to a limited number of seeds, experiments were carried out only under Zn- and Fe-deficient conditions with three replications for each genotype. Plants were grown for 21 d (Zn-deficient plants) and 13 d (Fe-deficient plants) in nutrient solution under controlled climatic conditions (light/dark regimes of 16/18 h, temperature 24/20 °C and photosynthetic photon flux of 350 μmol m^{-2} s^{-1} at plant height provided by Osram Sylvania cool white FR 96 T12 tubes, Ontario-Canada).

Collection of root exudates and measurement of PS

On days 10, 14, 18, and 21 for Zn-deficient plants and 9, 11 and 13 for Fe-deficient plants root exudates were collected for the measurement of PS release from roots. For the collection of root exudates, intact plants were removed from the nutrient solution 2 h after the onset of the light period, and then the roots were repeatedly washed in deoinized water and transferred to 500 ml aerated deionized water for 3 h. After the collection of root exudates, Micropur (Roth GmbH, Karlsruhe-Germany) was added into root exudates (10 mg l^{-1} of root exudate) to prevent microbial degradation of phytosiderophores. Thereafter, exudate solutions were filtered through course filter paper and concentrated to 20 ml at 50 °C under vacuum for Cu-mobilization test and HPLC analysis.

The amount of PS in the root exudates was determined indirectly by measurement of Cu mobilized from a Cu-loaded resin (Chelite-N, diameter 0.05–0.1 mm, Serva, Heidelberg, Germany) and calculated as mobilized Cu equivalents per plant or g^{-1} dry wt. Preparation of the Cu-loaded resin was described elsewhere (Cakmak *et al*., 1996*b*). Separation and identification of PS were achieved by HPLC on resin-based anion exchange columns using gradient eluation with aqueous NaOH (Neumann *et al*., 1999).

Shoot and root concentrations of Zn and Fe

At harvest, plants were separated into roots and shoots and washed several times with deionized water. After drying at 70 °C, samples were ground and ashed at 550 °C for 8 h. The ash was dissolved in 3.3% (v/v) HCl, and Zn and Fe concentrations were determined by atomic absorption spectrometry.

Results

Plant growth and PS release under Zn deficiency

Average shoot dry weights of hexaploid and tetraploid wheats were very similar under Zn deficiency (Table 2). However, the genotypes within each species showed greater variation regarding shoot dry weight. Also, average root dry weights of diploid, tetraploid and hexaploid wheats were similar, but differed between genotypes of each species, particularly in the case of *T. dicoccum* (Table 2). Among all genotypes of wheat species, FAL-02 showed the greatest shoot and root dry weights. *Aegilops tauschii* accessions had the lowest shoot and root dry weight. Visual Zn deficiency symptoms, such as reduction in plant height and appearance of whitish-brown necrotic patches on leaves, developed first in tetraploid wheats (BBAA). These symptoms appeared after 10 d of growth and became more severe with time. Of all *T. dicoccum* genotypes, FAL-02 was the least affected genotype as judged from the severity of visual Zn deficiency symptoms. In hexaploid and diploid wheats, Zn deficiency symptoms appeared 2 or 3 d later than that of tetraploid wheats and developed slightly. *Aegilops tauschii* is a wild wheat species and had a slower growth rate compared to the primitive and cultivated wheats. Therefore, in the *Ae. tauschii* accessions Zn deficiency symptoms developed slightly; however, at the end of the experiment *Ae. tauschii* accessions were also severely affected by Zn deficiency. Zinc concentrations of shoots and roots were very low, ranging between 7–9 mg kg^{-1} dry wt for shoots and 8–12 mg kg^{-1} dry wt for roots (Table 2).

There was a highly significant positive correlation ($R^2 = 0.89$) between HPLC analysis and the results of the

Table 2. *Shoot and root dry matter production and Zn concentrations in shoots and roots of diploid, tetraploid and hexaploid wheat and* Aegilops tauschii *genotypes grown 21 d in nutrient solution without Zn supply*

Species/genotypes	Dry weight (mg $plant^{-1}$)		Zn concentrations (mg Zn kg^{-1} dry wt)	
	Shoot	Root	Shoot	Root
T. monococcum (AA)				
FAL-30	56 ± 1	32 ± 1	7.9 ± 1.0	10.9 ± 0.9
FAL-43	51 ± 4	32 ± 3	6.2 ± 0.8	7.8 ± 1.1
FAL-67	47 ± 8	26 ± 6	7.5 ± 1.6	7.8 ± 0.8
T. dicoccum (BBAA)				
FAL-13	59 ± 6	29 ± 4	8.7 ± 0.7	11.8 ± 0.4
FAL-21	65 ± 6	19 ± 4	9.1 ± 0.4	9.9 ± 0.3
FAL-02	90 ± 5	56 ± 3	7.3 ± 0.5	11.2 ± 0.4
T. aestivum (BBAADD)				
Bezostaja	66 ± 5	34 ± 4	7.1 ± 0.7	8.1 ± 0.3
T. compactum (BBAADD)				
Stammbaum	81 ± 6	30 ± 4	7.4 ± 0.7	7.5 ± 1.0
Aegilops tauschii (DD)				
400682	26 ± 1	11 ± 1	9.5 ± 0.2	8.3 ± 1.2
400630	41 ± 4	15 ± 1	6.8 ± 1.3	7.5 ± 0.5

Cu-mobilization test (Fig. 1), indicating this test as a simple and rapid method for PS analysis in root exudates. Therefore the amounts of PS released from roots were measured by this method. According to HPLC analysis, all genotypes of *Aegilops tauschii* and *Triticum* species used released only one PS, namely 2′-deoxymugineic acid, under both Zn and Fe deficiencies.

Under Zn-deficient conditions, the rate of PS release greatly varied depending on wheat genomes and plant age (Table 3). During the 21 d of growth under Zn deficiency, the rate of PS release showed first a slight increase and then decreased and remained at a low level in tetraploid wheats and *Ae. tauschii* accessions. In the diploid and, particularly, hexaploid wheats the rate of PS release was high. While Bezostaja was an exception, PS release generally increased with the development of Zn deficiency symptoms (Table 3). When the rate of PS release was expressed per unit root dry weight, hexaploid and tetraploid wheats showed the highest and lowest rate of PS release, respectively. Based on the PS release per root dry weight, hexaploid wheats released about 14-fold more PS from roots than tetraploid wheats (Table 3).

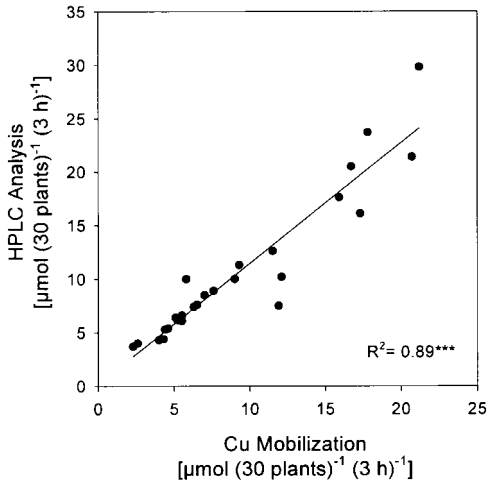

Fig. 1. Correlation between HPLC analysis and Cu-mobilization test for measurement of PS in root exudates collected from *Triticum* and *Aegilops* species under Fe deficiency ($n = 28$).

Among the genotypes of each species there were differences in rate of PS release. For example, among the *T. dicoccum* genotypes, FAL-02 showed the highest rate of PS release, and among the hexaploid wheats primitive wheat *T. compactum* released higher amounts of PS than the modern hexaploid wheat Bezostaja. Of the *Ae. tauschii* accessions, 400682 was superior to 400630 in rate of PS release (Table 3).

Plant growth and PS release under Fe deficiency

Under Fe deficiency, leaf chlorosis appeared on days 7 to 8 of growth in nutrient solution. Development of leaf chlorosis was observed first in the genotypes of tetraploid wheats, followed by the genotypes of other wheat species and *Ae. tauschii*. Average shoot dry weights of diploid, tetraploid and hexaploid wheats were similar under Fe deficiency, i.e. 24, 29 and 31 mg per plant, respectively (Table 4). However, within a given species genotypes tended to be different in their shoot dry weight, especially in the case of *T. dicoccum*. Among all species *Aegilops tauschii* showed the lowest shoot and root dry weight. Shoot Fe concentrations of plants under Fe deficiency were similar between *Triticum* and *Ae. tauschii* species, and ranged from 44 mg kg^{-1} dry wt (*T. dicoccum* and *T. tauschii*) to 49 mg kg^{-1} dry wt (*T. monococcum* and *T. aestivum*) (data not shown).

When compared with Zn deficiency (Table 3), Fe deficiency caused greater increases in rate of PS release

Table 3. *Effect of Zn deficiency on the rate of PS release from roots of diploid (AA), tetraploid (BBAA) and hexaploid (BBAADD) wheat and* Aegilops tauschii *(DD) genotypes during 21 d of growth in nutrient solution without Zn supply*

Results are means ± SD of three independent replications.

Species/genotypes	10-d-old [µmol (30 plants)$^{-1}$ (3 h)$^{-1}$]	14-d-old [µmol (30 plants)$^{-1}$ (3 h)$^{-1}$]	18-d-old [µmol (30 plants)$^{-1}$ (3 h)$^{-1}$]	21-d-old [µmol (30 plants)$^{-1}$ (3 h)$^{-1}$]	21-d-old [µmol g^{-1} root DW (3 h)$^{-1}$]
T. monococcum (AA)					
FAL-30	1.4 ± 0.2	3.2 ± 0.7	4.2 ± 0.7	3.7 ± 2.5	3.9 ± 2.7
FAL-43	0.7 ± 0.4	2.2 ± 0.2	2.5 ± 0.5	4.2 ± 0.8	4.3 ± 0.6
FAL-67	0.7 ± 0.5	2.8 ± 1.5	4.2 ± 1.7	4.0 ± 1.9	5.0 ± 1.8
T. dicoccum (BBAA)					
FAL-13	0.9 ± 0.1	1.8 ± 0.2	1.0 ± 0.2	1.0 ± 0.4	1.2 ± 0.4
FAL-21	1.9 ± 0.7	2.8 ± 0.6	0.8 ± 0.4	0.3 ± 0.2	0.7 ± 0.4
FAL-02	1.7 ± 0.7	1.9 ± 0.2	2.5 ± 0.6	1.9 ± 1.2	1.0 ± 0.5
T. aestivum (BBAADD)					
Bezostaja	5.4 ± 2.5	8.4 ± 0.7	8.6 ± 0.8	8.3 ± 3.1	8.2 ± 3.3
T. compactum (BBAADD)					
Stammbaum	4.0 ± 0.7	9.3 ± 0.6	11.5 ± 0.4	13.5 ± 1.6	15.2 ± 2.0
Aegilops tauschii (DD)					
400682	1.6 ± 0.9	4.8 ± 1.2	5.3 ± 1.2	1.0 ± 0.6	3.1 ± 1.9
400630	1.4 ± 0.4	2.4 ± 0.3	1.2 ± 0.5	0.7 ± 0.3	1.6 ± 0.7

during 13 d of growth, especially per unit of root dry weight (Table 5). On day 13, the highest rate of PS release expressed per 30 plants was found in hexaploid wheats, followed by tetraploid and diploid wheats and *Ae. tauschii*. In contrast to the results obtained under Zn deficiency (Table 3), rates of PS release were, on average, higher in tetraploid wheats than those of diploid wheats (Table 5).

Discussion

When grown under non-stressed conditions, tetraploid wheats (BBAA) generally produce highest shoot dry weight, followed by hexaploid (BBAADD) and diploid wheats (AA) (Bamakramah *et al*., 1984; Cakmak *et al*., 1999*b*). However, under Zn-deficient conditions, a decrease in shoot growth is much greater in tetraploid wheats than diploid and hexaploid wheats, indicating higher sensitivity of tetraploid wheats to Zn deficiency. Readers are referred to Cakmak *et al*. for detailed information about the effects of varied Zn supply on shoot dry matter production of diploid, tetraploid and hexaploid wheats in Zn-deficient calcareous soils (Cakmak *et al*., 1999*b*).

Compared to hexaploid wheats (BBAADD), tetraploid wheats (BBAA) released much less PS under Fe and, particularly, Zn deficiency (Tables 3, 5). This result is in agreement with the results obtained with different tetraploid and hexaploid wheats (Cakmak *et al*., 1994, 1996*b*; Rengel *et al*., 1998; Rengel and Römheld, 2000*a*). The low rate of PS release under Zn deficiency was considered as an important reason for high sensitivity of tetraploid wheats to Zn deficiency. As was also observed in the present study, tetraploid wheats appeared to be more sensitive to Fe deficiency than hexaploid wheats (Rengel and Römheld, 2000*b*). Release of PS at lower rates might be one factor contributing to the higher sensitivity of tetraploid wheats to Fe deficiency compared to hexaploid wheats. However, the role of PS in expression of high tolerance to Zn or Fe deficiency should be evaluated carefully. It has also been reported that bread wheat cultivars showing very high sensitivity to Zn deficiency, like durum wheats, released PS at rates as high as those of the Zn deficiency-tolerant bread wheat cultivars (Erenoglu *et al*., 1996; Cakmak *et al*., 1998).

Diploid wheats (AA) are very tolerant to Zn deficiency when grown in Zn-deficient calcareous soils (Cakmak *et al*., 1999*b*), and the transfer of the whole A genome from diploid wheat *T. monococcum* to tetraploid wheat (BBAA) *T. turgidum* markedly enhanced tolerance to Zn deficiency and improved growth under Zn deficiency (Cakmak *et al*., 1999*d*). Despite their high tolerance to

Table 4. *Shoot and root dry matter production of diploid, tetraploid and hexaploid wheats and* Aegilops tauschii *grown in nutrient solution with low Fe supply (1 μM) for 13 d*

Results are means ± SD of three independent replications.

Species/genotypes	Dry weight (mg plant^{-1})	
	Shoot	Root
T. monococcum (AA)		
FAL-30	28 ± 1	10.8 ± 0.6
FAL-43	21 ± 1	7.5 ± 0.0
FAL-67	23 ± 2	10.8 ± 0.8
T. dicoccum (BBAA)		
FAL-13	26 ± 1	9.2 ± 0.8
FAL-21	23 ± 1	9.0 ± 0.5
FAL-02	38 ± 3	14.7 ± 2.8
T. aestivum (BBAADD)		
Bezostaja	34 ± 4	11.8 ± 1.3
T. compactum (BBAADD)		
Stammbaum	28 ± 2	8.3 ± 0.8
Aegilops tauschii (DD)		
400356	15 ± 1	4.5 ± 1.3

Table 5. *Effect of Fe deficiency on the rate of phytosiderophore release from roots of diploid (AA), tetraploid (BBAA) and hexaploid (BBAADD) wheats and* Aegilops tauschii *(DD) during 13 d of growth in nutrient solution with low Fe supply (1 μM)*

Results are means ± SD of three independent replications.

Species/genotypes	9-d-old [μmol (30 plants)$^{-1}$ (3 h)$^{-1}$]	11-d-old [μmol (30 plants)$^{-1}$ (3 h)$^{-1}$]	13-d-old [μmol (30 plants)$^{-1}$ (3 h)$^{-1}$]	13-d-old [μmol g^{-1} root DW (3 h)$^{-1}$]
T. monococcum (AA)				
FAL-30	3.2 ± 0.4	7.6 ± 0.7	6.7 ± 1.1	20.6 ± 2.7
FAL-43	2.9 ± 0.3	3.9 ± 0.5	5.2 ± 0.7	23.2 ± 3.2
FAL-67	3.4 ± 0.3	5.2 ± 0.9	5.3 ± 1.1	16.2 ± 2.3
T. dicoccum (BBAA)				
FAL-13	3.4 ± 0.4	6.0 ± 0.2	5.0 ± 0.4	18.5 ± 2.7
FAL-21	4.7 ± 0.6	6.2 ± 0.9	8.2 ± 1.7	30.3 ± 5.1
FAL-02	5.8 ± 0.1	4.8 ± 0.3	11.9 ± 0.4	28.0 ± 6.7
T. aestivum (BBAADD)				
Bezostaja	9.5 ± 1.2	16.5 ± 0.5	18.3 ± 2.7	51.7 ± 6.6
T. compactum (BBAADD)				
Stammbaum	7.0 ± 0.6	11.1 ± 0.6	11.8 ± 0.3	47.6 ± 3.7
Aegilops tauschii (DD)				
400356	2.3 ± 0.5	2.3 ± 0.5	3.0 ± 0.9	22.0 ± 2.6

Zn deficiency, diploid wheats (AA) did not release PS from roots at rates as high as those of hexaploid wheats (BBAADD) (Table 3). Even under Fe deficiency, diploid wheats generally released much less PS as compared with tetraploid and hexaploid wheats (Table 5). Therefore, it can be suggested that high tolerance of diploid wheats to Zn deficiency is not directly related to release of PS from roots, as proposed previously (Cakmak *et al*., 1999*b*, *d*). The reason for high tolerance of diploid wheats to Zn deficiency needs to be explained by mechanisms other than PS release, such as root uptake of Zn and its distribution and/or cellular compartmentation within plants.

Similar to diploid wheat genotypes, *Ae. tauschii* (DD) genotypes were not effective in the release of PS from roots under either Zn or Fe deficiency. These results suggest that the D and A genomes alone cannot solely contribute to the release of PS from the roots of wheats under Fe or Zn deficiency. When expressed per plant, the rate of PS release in wheat species under Fe deficiency decreased in the order:

T. aestivum (BBAADD) > *T. dicoccum* (BBAA) > *T. monococcum* (AA) > *Ae. tauschii* (DD)

Recently, the same order has been shown (Ma *et al*., 1999) under Fe deficiency using only one genotype from each wheat species. However, in the case of Zn deficiency, the order of PS release was not similar to that found under Fe deficiency. PS release under Zn deficiency decreased in the following order:

T. aestivum (BBAADD) > *T. monococcum* (AA) > *Ae. tauschii* (DD) > *T. dicoccum* (BBAA)

All wheat genomes carry genes affecting the biosynthesis and release of PS from roots. However, these genes are expressed predominantly and much more effectively when three genomes A, B and D are present together. This indicates the importance of complementary action of the corresponding genes from A, B and D genomes to activate the biosynthesis and release of PS in wheat. All these genomes were, to different extents, responsible for production of only one phytosiderophore, 2′-deoxymugineic acid (DMA). This result is in full agreement with the results published earlier (Ma *et al*., 1999). 2′-Deoxymugineic acid acts as a key precursor in biosynthesis of other phytosiderophores and is the major PS in root exudates of wheats (Mori *et al*., 1990; Ma and Nomoto, 1996).

When compared with tetraploid wheats (BBAA), diploid wheats (AA) released higher PS under Zn deficiency (Table 3). This result may suggest that the A genome has a greater importance than the B genome in the release of PS under Zn deficiency. Since tetraploid wheats with high sensitivity to Zn deficiency (Graham *et al*., 1992; Cakmak *et al*., 1996*a*; Rengel and Römheld, 2000*b*) consistently showed a low rate of PS release in a number of studies (Table 2; Cakmak *et al*., 1994, 1996*b*; Walter *et al*., 1994; Erenoglu *et al*., 1996; Rengel *et al*., 1998; Rengel and Römheld, 2000*a*) it might be suggested that the B genome of tetraploid wheats possess suppressor genes under Zn-deficient conditions; these suppressor genes possibly repress expression of the genes affecting biosynthesis and release of PS. This effect of Zn deficiency seems to be specific, because in the case of Fe deficiency, the rate of PS release from roots of tetraploid wheats is still fairly high and comparable with those found with hexaploid wheats. In future, studies should focus on a better understanding of the roles of the A, B and D genomes in the release of PS under Zn-deficient conditions by using tetraploid wheats with added A or D genomes (synthetic wheats) and accessions of *Ae. speltoides* and *Ae. searsi* which are considered the most probable donors of the B genome to tetraploid (BBAA) and hexaploid (BBAADD) wheats (Feldmann and Kislev, 1977; Kerby and Kuspira, 1987; Daud and Gustafson, 1996).

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