Aus dem Institut für Pflanzenernährung
der Universität Hohenheim
Prof. Dr. Volker Römheld

PHYSIOLOGICAL DIFFERENCES IN THE RESPONSE OF CEREALS TO ZINC DEFICIENCY

Dissertation
zur Erlangung des Grades eines Doktors
der Agrarwissenschaften

der Fakultät III-Agrarwissenschaften I-
Pflanzenproduktion und Landschaftsökologie

von

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3. Prüfer	: Prof. Dr. E. Kandeler

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1. General Introduction

Zinc (Zn) deficiency has been recognized as a common nutritional problem in soils and occurs predominantly in calcareous soils of arid and semiarid regions and heavily weathered soils of tropics and subtropics. In a worldwide study introduced by the FAO, it was mentioned that almost 50% of soil samples collected from arable soils in 25 countries have low Zn availability (Sillanpää, 1990). According to Graham and Welch (1996), in particular, cereal growing areas suffer from low Zn availability. In about 50% of the cereal producing areas (mainly wheat) Zn deficiency causes severe reductions in grain yield and also in nutritional quality of the grains.

Soil and foliar applications of Zn fertilizers are common methods used by farmers to correct Zn deficiency and increase crop production. However, these methods are expensive, time-consuming and effective only for one or two cropping seasons, probably, because of high Zn fixation capacities of soils with high pH (Shuman, 1975; Perlata et al., 1981), clay (Shuman, 1975, 1976) or $CaCO_3$ content (Udo et al., 1970). Alternatively, selection and breeding of plant genotypes with a high capacity to acquire Zn from soils are more sustainable solution to the Zn deficiency problem in crop plants and humans. This approach is especially reasonable in view of the existence of large genotypical differences in cereals in resistance to Zn deficiency. Only in recent years, promising progress has been made in the characterization and selection of Zn-efficient cereal genotypes, mainly in Australia (Graham et al., 1992; Graham and Rengel, 1993) and Turkey (Cakmak et al., 1996a, d, 1998).

Crop species markedly differ in their ability to adapt to soils low in Zn availability. Among the cereal species, rice, sorghum, and maize are classified as species sensitive to Zn deficiency, whereas barley, wheat, and rye are classified as less sensitive (Clark, 1990). Similarly in a more recent study, it was shown that resistance to Zn deficiency of cereals grown on a Zn-deficient calcareous soil increases in the order: rye, triticale, barley, bread wheat, oat, and durum wheat (Cakmak et al., 1998).

In recent years, the variation of Zn efficiency within different cereal genotypes has attracted extensive attention. 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, release of Zn-mobilizing phytosiderophores (PS's), uptake, translocation, and compartmentation of Zn (Graham and Rengel, 1993). Later on, 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 PS's from roots (Cakmak et al., 1994, 1996c, 1998), and an increased Zn uptake capacity of roots (Rengel and Graham, 1996; Cakmak et al., 1998) were suggested as main parameters determining Zn efficiency. Even particular enzymes were considered as important indicators for the internal utilization efficiency of Zn at the cellular level, such as carbonic anhydrase (Rengel, 1995) and Cu-Zn-SOD (Cakmak et al., 1998). In a recent review Rengel (2000) mentioned that in the case of wheat grown on low Zn soils in the Australia, mycorrhiza did not ameliorate Zn deficiency since (i) natural infection of VA mycorrhizae is generally low (Graham & Rengel, 1993) and (ii) in soils supplied with sufficient P, mycorrhizal infection is usually almost absent (Baon et al., 1992). In contrast, in Zn-deficient soils of Turkey, higher mycorrhizal infection rates were determined for cereal roots, even in plants supplied with sufficient P (Ortas, personal communication).

The main goal of this PhD thesis was to verify the following selected mechanisms which have been discussed in literature as possible determinants for differential Zn efficiency in cereals. In the first part of this PhD thesis, a short review on Zn in soils and plants is given (***Chapter*** **2**). While the first section in this chapter focused on Zn in soils, the chemical forms in soils, and soil or environmental factors influencing Zn availability, the second section dealt with the function of Zn in plants, critical tissue concentrations and deficiency symptoms, as well as Zn uptake and translocation. Furthermore, evidence for genotypical variations and possible physiological mechanisms effective on Zn efficiency was presented.

In the second part of the thesis (***Chapter*** **3** and **4**), two soil experiments were discussed that were conducted on a soil with extremely low Zn availability, imported from Central Anatolia in Turkey. The first experiment aimed to compare growth performances of one rye, two bread wheat, and one durum wheat cultivars on this Zn-deficient soil and the results of this experiment were evaluated using different parameters reflecting Zn efficiency. ***Chapter*** **4** presents an investigation on the possible role of VAM in Zn uptake and Zn efficiency of wheat cultivars differing in Zn efficiency.

In the third part of this PhD thesis (***Chapter*** **5** and **6**), the contribution of PS release to the expression of Zn efficiency of cereals was described. In ***Chapter*** **5**, Zn efficiency and PS release were correlated for several different cereal species. For this purpose, a new assay for the indirect determination on Zn-mobilizing root exudates was tested. The aim of the work in ***Chapter*** **6** was to measure the rate of PS release in bread wheat cultivars that show large differences in the severity of visual Zn deficiency symptoms when grown on Zn-deficient soils in Turkey.

In a following part (***Chapter*** **7**), the contribution of Zn uptake by roots and root-to-shoot translocation of Zn to differential Zn efficiency was compared in one rye, three bread wheat, and one durum wheat cultivars by using radio-labeled Zn (^{65}Zn).

In last part of the thesis (***Chapter** 8* and *9*), the relationship between phloem mobility (retranslocation) and Zn efficiency of bread and durum wheat cultivars was elucitated. In ***Chapter** 8*, the movement of ^{65}Zn applied to the tip of oldest leaf, from old leaves to different plant parts (young leaves, old leaves, and roots) was monitored during a defined growth period in nutrient solution culture, and the significance of this translocation for Zn efficiency was discussed. To approach natural conditions, plants were grown with ^{65}Zn during preculture and Zn retranslocation was observed (***Chapter** 9*).

2. Literature Review

2.1. Zinc in soils

The upper continental crust has a Zn concentration more or less 57 μg g^{-1} (Brehler & Wedepohl, 1978). The results of the analysis of selected soils from 18 different countries from all continents show that the concentration of Zn ranges from 10 to 300 μg g^{-1} with an approximate average value of 70 μg g^{-1} (Swaine, 1955).

One of the most important determinants for the solubility relationships of a nutrient in soils is the solid phases. The precipitation of a mineral can occur whenever the equilibrium concentration of that mineral is exceed by a nutrient element in the soil solution. On the other hand, whenever the concentration of a nutrient in the soil solution decreases below the equilibrium solubility of a solid phase, that solid can dissolve. Thus, the solubility of nutrients in soils is buffered by solid phases and, in this case, solid phases directly affect the availability of plant nutrients (Lindsay, 1991).

The manner in which inorganic solid phases tend to interact with other components of soil is given graphically in *Figure 2.1*. When the nutrients are absorbed from solution by plants (*Reaction 1*), the concentrations of nutrients next to the absorbing roots drop. Nutrients present on the soil exchange sites flow into solution (*Reaction 4*) to replenish those being depleted. Dissolution of solid phases which restores dropped ions from solution and replenishes them on soil exchange sites (*Reaction 3*) occurs when the nutrients are removed from solution. Nutrients are also removed from solution by microorganisms in the run of their life cycles (*Reaction 4*). The decomposed organic matter is also a good source of nutrients (*Reaction 8*) (Lindsay, 1991).

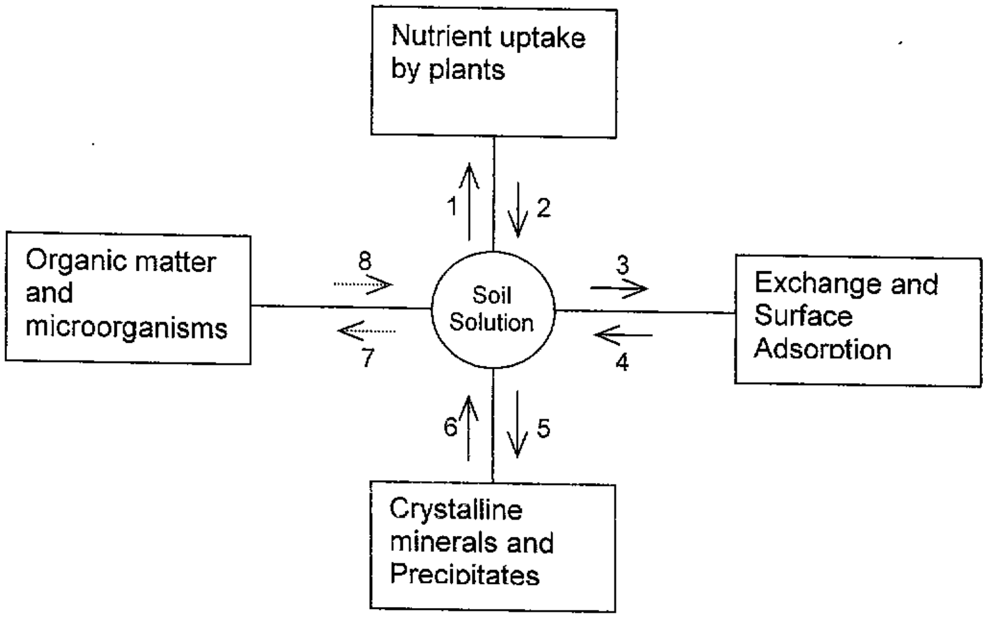


Figure 2.1. Dynamic equilibria in soils (Lindsay, 1991)

2.1.1. Chemical forms of zinc in soils

The main types of Zn(II) minerals are sulfides (ZnS, sphalerite and wurtzite), sulfates ($ZnSO_4$, zincosite; $ZnSO_4 \cdot 2H_2O$, goslarite), oxides (ZnO, zincite; $ZnFe_2O_4$, franklinite; $ZnAl_2O_4$, gahnite), carbonates (smithsonite, $ZnCO_3$), phosphates ($Zn_3(PO_4)_2 \cdot 4H_2O$, hopeite), and silicates (Zn_2SiO_4, willemite; $Zn_4Si_2O_7(OH)_2 \cdot H_2O$, hemimorphite). Among these minerals, since ZnO, Zn-hydroksite (amorp), $Zn_3(PO_4)_2 \cdot 4H_2O$ and $ZnCO_3$ are very soluble, they do not exist in solid phases. Willemite has moderate solubility and possibly because of this reason, it plays an important role in the solubility of Zn in soils. The solubility of franklinite is very low and since it contains both Zn(II) and Fe(III), so the solubility of this Zn^{2+} controlled by this mineral is also affected by the solubility of Fe^{3+} (Lindsay, 1991).

Shuman (1991) had mentioned that micronutrients and Zn, too, in soil could exist (*I*) as complexed with both inorganic and organic ligands in soil solution (most of the metallic micronutrients in soil solution are not in a free ionic form), (*II*) as adsorbed on surface and exchangeable, (*III*) as associated with organic matter, (*IV*) as associated with oxides, and (*V*) as fixed in primary minerals and secondary alumino-silicate minerals.

Certainly the soil solution is the center of soil chemistry, since plants take nutrients up from this space and it is the center of all important soil processes (Lindsay, 1979). Element concentrations in soil solution are in constant flux, influenced by a host of factors including moisture, pH, temperature, oxidation/reduction status, fertilizer additions, and plant uptake (Shuman, 1991). There is increasing evidence that the free hydrated cationic form of zinc and other trace elements is only dissolved form that is actively assimilated by plant roots, although complexed forms of dissolved zinc may be active in transporting zinc to the plant roots (Barak & Helmke, 1993 and references therein). In soil solution, below pH 7.7, Zn^{2+} is the most dominant Zn species and above this pH, $ZnOH^{+}$, and eventually $Zn(OH)_2^0$ become dominant. Zinc solubility is closely related with soil pH and the solubility of Zn can be increased hundred-fold with one unit increase in pH (Lindsay, 1991).

According to the results of experiment conducted by using physical and chemical methods to fractionate soils, it can be easily expressed that zinc in the solid phase of soils is associated with all of the widespread soil solid phases; including clay minerals, hydrous oxides, and organic matter (Barak & Helmke, 1993). In just the same way, after the sequential extraction of soils, Hickey and Kittrick (1984) expressed that Zn could be partitioned into five operationally defined geochemical fractions: exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter, and residual.

Most of total soil-Zn is in unavailable forms. That is to say, the amount of plant available forms such as water soluble, exchangeable and organic forms, are less than oxides and residual forms (Emmerich et al., 1982; Berthet et al., 1984). According to LeClaire et al. (1984), the water soluble and exchangeable tools of Zn are accepted as very bio-available, carbonate is potentially available, and the residual are non-available. Levesque and Mathur (1988) concluded that the sum of water-soluble and exchangeable fractions may constitute the basis of a good soil test for available Zn, according to results of experiment in which they related both fractions to the intensity factor. DTPA-extractable Zn, as well as other extractable forms, are often correlated with the more mobile fractions, such as exchangeable and organic fractions, and thus, are thought as plant available (Shuman, 1986, 1988b).

2.1.2. Zinc adsorption-desorption in soils

Adsorption is a major contributing factor to low concentrations of Zn in solution in soils with low Zn availability (Ellis & Knezck, 1972). Harter (1983) mentioned that Zn is probably retained on nonspecific, exchange complex sites throughout the pH range of soils. It was shown that soil-Zn could be effective in Zn adsorption, either by changing the number of sites available for adsorption or by changing the concentration of the Zn species that is preferentially adsorbed (Barrow, 1986). Soluble organic ligands may also affect Zn adsorption by these two mechanisms. Adsorption of organic anions can increase the negative charge on surfaces (Barrow, 1985) and hence increase Zn adsorption. Whatever the mechanism(s) involved, it was mentioned by Christensen (1988) that pH is the most important property in controlling Zn mobility. Among the other properties which are effective in Zn adsorption, clay minerals (Reddy & Perkins, 1974; Shuman, 1975, 1976; Kabata-Pendias, 1980; Tiller et al., 1984a, b), hydrous oxides (McKenzie, 1972; Forbes et al., 1976; Bolland et al., 1977; Shuman, 1977; Barrow et al., 1981, 1986; Ghanem & Mikkelsen, 1988), and organic matter (Shuman, 1980; Kabata-Pendias & Pendias, 1984) could be given.

Zinc desorption reactions play a very important role in determination of Zn amount in soil solution and the availability to plants. Generally, most of the applied Zn, after a few days of reaction period, is adsorbed. Since Zn is needed for a better plant growth, desorption rate is very considerable in regulation of plant availability of this nutrient. Two chelating agents, DTPA and EDTA, are routinely used to estimate available Zn in soil (Lindsay & Cox, 1985). But they provide only a potential information on Zn availability to plants.

2.1.3. Factors effective on availability of zinc

There is only poor relation between the total amount of micronutrients in soils and availability of them to plants. Various soil properties such as pH, redox potential, amount of organic matter, nutrient interactions, plant species or cultivars, and environmental factors, such as soil water content, temperature and light, greatly affect the possibility that a plant will show micronutrient deficiency or toxicity symptoms. Environmental changes influence the micronutrient nutrition of plants more than macronutrient nutrition (Moraghan & Mascagni, 1991).

2.1.3.1. Environmental Factors

Temperature and light affect the result and severity of Zn deficiency in crop plants. On the other hand, soil humidity besides its indirect effect on normal growth and nutrient requirement, possibly has no important effect on Zn deficiency in non-flooded crops grown in field (Moraghan & Mascagni, 1991). Although the evidence is opposition, it was concluded that metabolic control mechanisms are probably responsible for absorption of Zn (Moore, 1972; Mengel & Kirkby, 1987). As a result of this, if root temperature increases, Zn uptake should be enhanced. Difficulties in separation of Zn adsorption onto root or cell surfaces from real Zn uptake may cause problems in Zn-uptake studies (Schimid et al., 1965; Chaudhry & Loneragan, 1972).

Soil temperature: Some researches conducted under controlled temperature conditions pointed out that Zn deficiency is of a great degree under low soil-temperature conditions (corn–Giordano & Mortvedt, 1978; tomato–Martin et al., 1965; flax-Moraghan, 1980). Results obtained by Martin et al. (1965) show clearly following general interpretations from such work: (i) more severe Zn-deficiency symptoms at a low soil temperature; (ii) low probability to respond to Zn fertilization at a high soil temperature; (iii) probably more P-induced Zn deficiency at a low soil temperature; and (iv) increased Zn concentration and uptake at a high soil temperature.

It was expressed by Edwards and Kamprath (1974) that the damaging effect of low root temperature on Zn accumulation by corn in a solution culture experiment was in some degree related with lowered translocation from roots to shoot. Similar observations, accumulation of adsorbed or absorbed Zn in the root system at lower soil temperatures were done for barley (Schwarz et al., 1987). One of the possible reasons of the restricted Zn uptake at lower temperatures could be reduced root growth (Burleson et al., 1961).

Since foliar absorption is affected by air temperature rather than soil temperature, for crops growing in cold climates, the most effective application method is foliar fertilization (Sutcliffe, 1971).

It was pointed out that the uptake of Zn is increased by mycorrhizas (Singh et al., 1986; Kothari et al., 1991). Low soil temperatures are responsible for reduced vesicular-arbuscular (VA) mycorrhizal infection (Hayman, 1974). Consequently, reduced VA mycorrhizal infection may involved in low soil-temperature–induced Zn deficiencies.

Graminaceous plant species respond to deficiencies of Fe (Römheld, 1987; Takagi, 1976) and Zn (Cakmak et al., 1994; Zhang et al., 1989) with an enhanced release of phytosiderophores from roots. Release of phytosiderophores is also related to root zone temperature. It was shown that decrease in root zone temperature from 30 °C to 5 °C decreased the rate of release of phytosiderophores (Kissel, 1987). Because of this reason, it can be thought that the low root zone temperature is a limiting factor for release of phytosiderophores and so causes decrease in Zn uptake by plants.

Soil Moisture: In some arid conditions, during most of the growing season, the zone with usually the largest amount of plant available Zn, the top soil, is dry (Follett & Lindsay, 1970). In such cases, plants can not do much to eliminate damaging effect of Zn deficiency, even though subsoil moisture is enough to support the growth. As most Zn is bound to the soil particles, the concentration in soil solution is generally very low and thus also the supply by mass flow the roots (Marschner, 1993). The main reach path of Zn to the roots is diffusion and at low soil moisture levels these diffusion paths become more tortuous (Warncke & Barber, 1972). Because of these reasons, according to Moraghan and Mascagni (1991) in such soil moisture levels, stronger Zn deficiency stress in susceptible crops would be awaited. After all, it could be mentioned that one of the most important soil factors which are affective in the movement (diffusion) of Zn to roots, is the content of the soil moisture.

Light: Changes in light quality, light intensity and daily exposure period may be effective on the seasonal nature of certain Zn deficiency problems (Moraghan & Mascagni, 1991). Low or intermediate light intensities cause more severe Zn deficiency stress in subterranean clover (Ozanne, 1955) and corn (Edwards & Kamprath, 1974). In the same study, Edwards and Kamprath (1974) showed that under reduced light intensities, total Zn content of the seed is very important for early growth of corn. Reduced root growth and decreased uptake of soil Zn under low light intensities were discussed as possible factors for this greater dependence on seed Zn.

It was also reported that under reduced light intensity, Zn deficiency sometimes become less severe (Cakmak et al., 1995; Marschner & Cakmak, 1989; Obata et al., 1997). Cakmak et al. (1995) demonstrated a major role of high light intensity in the development of low temperature induced leaf chlorosis in field-grown citrus plants and they dedicated this chlorosis to Zn deficiency. The response of subterranean clover grown during long days to Zn supply was much greater than the response of similarly treated short-day (7.5 h) plants (Ozanne, 1955). Hewitt (1984) advised that daily exposure period and light intensity should be considered in plant-Zn studies.

2.1.3.2. Soil Factors

The availability of Zn could be affected by various soil properties. Since, in soil solution, the concentrations of micronutrients are very low, relatively little plant growth is realized by the total quantity present at any time (Loneragan, 1975). For optimum growth, as the micronutrients depleted from the soil solution, they should be rapidly filled up from the soil solution again. As mentioned above, Zn moves to the plant root primarily by diffusion, since most Zn is bound to the soil particles. Because of this reason, soil properties that are effective on the mobility of Zn, will also affect the Zn availability. Parent material, pH, organic matter, (redox potential), and nutrient interactions are discussed as possible factors which can affect the availability of Zn in soil. The possible effective soil properties on Zn availability are given schematically in *Figure* 2.2 (adapted from Marschner ,1993).

Parent Material: Total Zn concentration of soils differs greatly. According to Aubert and Pinta's (1977) conclusion, soil Zn content is related to parent material more than to pedogenic factors. They showed that: basic eruptive rocks, such as basalt and gabbro, generally hold more Zn than acid eruptive rocks, metamorphic rocks, or sedimentary rocks, such as limestone or sandstone; and soil layers rich in organic matter, such as upper soil horizons contain higher Zn amounts. However, possibility of uniformly distribution of total Zn in soil profiles was also mentioned by other researchers (Follett & Lindsay, 1970). Karim and Sedberry (1976) expressed that it could be even concentrated in the subsoil.

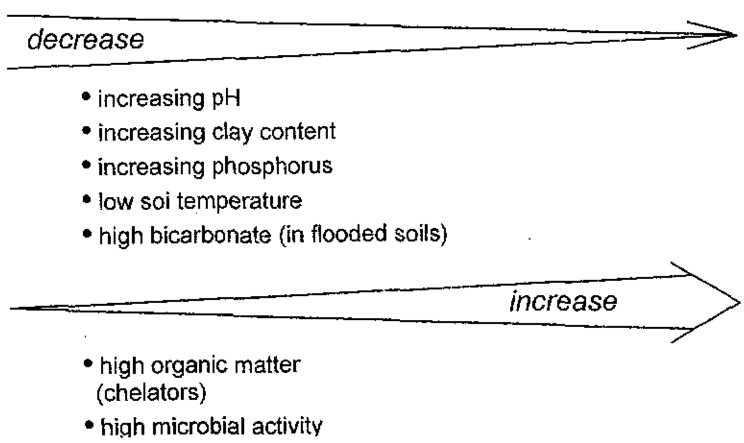


Figure 2.2. Main soil properties decreasing or increasing zinc availability and uptake by soil grown plants (Marschner , 1993)

The results obtained from most of the experiments show that plant available Zn locates in the top soil (Follett & Lindsay, 1970) or upper soil horizons (Aubert & Pinto, 1977).

The amount of quartz also affects the concentration of Zn in soils. Since Zn concentration is very low in quartz, ranging from 1.0 $\mu g\ g^{-1}$ (Helmke et al., 1977) to <5 to 8 $\mu g\ g^{-1}$ (Brehler & Wedepohl, 1978), soil Zn is diluted in the presence of quartz.

<u>Soil pH:</u> Among the soil factors, pH has the most clear influence on plant availability of Zn. In the pH range of 5.5-7.0, per unit increase in soil pH may cause 30-45 times decreases in the equilibrium concentration of Zn (Barber, 1984; Moraghan & Mascagni, 1991). As expressed before, diffusion is the main path of Zn transfer from soil solution to the root surface and it seems that this transfer occurs close to the root. The diffusion coefficient in a

calcareous loam was calculated approximately 50-fold less than in an acidic soil. Thus, Zn mobility is inhibited and plants suffer much from Zn deficiency on soils with high pH levels (Sedberry et al., 1980). Liming of acid soils makes possible a decrease in diffusion coefficient of Zn, similar to values found in calcareous soils (Moraghan & Mascagni, 1991). Zinc contents in plants are decreased exactly by liming acid soils (Parker & Walker, 1986). Although Zn content in plants decreases very sharply, the DTPA extractable Zn in soils is often not, or only slightly reduces (Lins & Cox, 1988) and according to Marschner (1993), in such case it could be suggested that the "rate" and "intensity" of Zn play a more important role in limiting the supply to rot surface than "capacity". Soil extraction methods respond less sensitively to changes in soil pH than the concentrations of Zn in soil solution and thus also diffusion coefficient and plant uptake (Marschner, 1993).

Decrease in plant-available Zn as soil pH increase is due partly to enhanced sorption by soil constituents (Bar-Yosef et al., 1980). As soil pH jumps above approximately 5.5, adsorption of Zn on hydrous oxides of Al (Kalbasi et al., 1978), Fe (Kinniburgh & Jackson, 1982), and Mn (Loganathan et al., 1977) occurs. In the pH range of 5.5-6.5, Zn adsorption increased by a factor of 10 at equilibrium Zn concentration of about 1 mol m^{-3} (Kinniburgh & Jackson, 1982). As mentioned above, this increase in adsorption of Zn reaches to about 45 times for each unit increase pH, showing more strongly pH dependency of Zn adsorption .

Excretion of H^+ and other acidifying processes in rhizosphere were discussed as more effective in enhancing Zn uptake than release of complexing agents (Bar-Yosef et al., 1980). One of the responses of Strategy I plants to Fe deficiency stress, release of H^+, and rhizosphere acidification from NH_4-N uptake of plant may encourage Zn uptake.

<u>*Soil Organic Matter*</u>: Soil organic matter occurs in various forms, including water-soluble and solids in various degrees of decomposition. Micronutrients are related with these materials by being incorporated in the structure of the less decomposed material, by chelation, which causes resistance to exchange, and by exchange and adsorption sites, both spesific and nonspecific. The most of the total Zn in soils is in the form of unavailable. Because of this reason, amount of Zn in more plant-available forms, in the water soluble, exchangeable, and organic, are lower than less plant-available forms such as the oxide and residual fractions (Berthet et al., 1984; Emmerich et al., 1982; Hickey & Kittrick, 1984).

The percentage of Zn found in the organic fraction generally is greater than that in exchangeable fraction (Sanders et al., 1986). There is a well relation between the amount of Zn in organic fraction and the amount of organic matter in soil (Mandal et al., 1986; McGrath et al., 1988). Organic matter addition increases the amount of applied Zn in all fractions, except the crystalline Fe oxides and the residual which indicates an increase in bioavailability (Mandal et al. 1988).

Manure application causes an increase in the water-soluble fraction of Zn, the form of highest bioavailable Zn (Singhania, 1983). Organic matter additions change the Zn distribution from the oxide to the organic fraction (McGrath et al., 1988).

<u>*Nutrient Interactions*</u>: Discussions on interactions between Zn and other nutrients affecting the growth of plants are mainly focused on Zn-P interaction. Many studies show that increasing P supply may enhance Zn deficiency. But this interaction between P content and Zn availability is very complex and confusing. As mentioned by Loneragen and Webb (1993), many phenomena in both soils and plants involve in this complex Zn-P interactions. These phenomena may be separated into two categories

according to whether increasing P applications decrease or not decrease Zn concentrations in shoot.

The early approach was P-induced Zn deficiency resulted from precipitation of Zn-phosphates ($Zn_3(PO_4)_2$) in soil or on the roots. Some researchers mentioned that in P applied certain soils, Zn adsorption could increase, and this may be the reason of appearance of Zn deficiency (Gnahem & Mikkelsen, 1988; Saeed & Fox, 1979). Saeed and Fox (1979) found that in the soils which they used, P additions increased Zn adsorption, particularly, in soils that contained higher amounts of hydrated Fe and Al oxides. However, later on, it was mentioned that the solubility of $Zn_3(PO_4)_2$ is more than sufficient to supply Zn to plants, $Zn_3(PO_4)_2$ is an effective Zn fertilizer (Lindsay, 1991), and higher P rates generally increase extractable soil Zn (Saeed, 1977).

In some cases, decreases in Zn concentration of plants by increasing P supply could simply be explained by a dilution effect (Loneragan et al., 1979; Singh et al., 1986). In other cases, increase in amount of P supply induced or enhanced Zn deficiency by reducing Zn concentrations in shoot more than can be expected by dilution from plant growth (Safaya, 1976). Here, according to Loneragan and Webb (1993), either Zn absorption by roots or Zn translocation from roots to shoots should have been dropped by P supply.

Since the diffusion of Zn to the root surface controls mainly Zn absorption by the plant roots, root volume should play a direct role in Zn absorption (Moraghan & Mascagni, 1991). Increasing total Zn uptake by enhanced P supply were attributed to a larger root absorbing surface as a result of improved P nutrition (Friessen et al., 1980). Reduction in VA mycorrhizal infection by P application may also reduce Zn application (Singh et al., 1986). On the other hand, while in both field and growth-chamber experiments P application reduced sharply VA mycorrhizal infection, Zn absorption was not affected (Lu & Miller, 1989).

Interactions involving in Zn and the other plant nutrients have also been discussed in plant nutrition studies (Moraghan & Mascagni, 1993 and references therein), but the importance of the P-Zn interactions is always greater than the others under most conditions. And these studies were done so many years ago and special interest should be given in future studies.

2.2. Zinc in plants

The first observation of the biological role of Zn was done by Raulin in 1869. He found that the absence of Zn did not let common bread mold (*Aspergilus niger*) grow. After a short time, Zn was accepted as an ubiquitous component of both animal and plant tissue. This identification opened the ways to Zn research in crops, and in 1914 Zn deficiency in plants was shown firstly. In 1926 (Sommer & Lipman), the general essential of Zn was evident. The first demonstration of Zn in field conditions was done by Chandler (1937). Now, Zn deficiency is known as one of the most common micronutrient deficiencies all over the world.

2.2.1. Forms and function of zinc in plants

Like in other biological systems, Zn presents only as Zn (II) in plants, and does not join to oxidoreduction reactions. The metabolic functions are closely related with its strong tendency to form tetrahedral complexes with N-, O-, and particularly S-ligands and, by that, it plays both a functional (catalytic) and structural role in enzyme reactions (Vallee & Auld, 1990). In recent years, researches on the role of Zn in protein molecules involved in DNA replication and the regulation of gene expression were very attractive for the scientists (Coleman, 1992).

2.2.1.1. Forms of zinc in plants

Most of Zn in the leaf is combined with low molecular weight complexes, storage metalloproteins, free ions, and insoluble forms associated with the cell wall. Either ligand formation (Leece, 1978) or complexation with (Olsen, 1972) are the major ways for Zn to be inactivated within the cell. From 58% to 91% of plant Zn are water-soluble and it differs from one species to another one (Peterson, 1969; Welch et al., 1976). This fraction of Zn is generally accepted as the physiologically active fraction and is considered as a better indicator of Zn nutritional status than total Zn content (Cakmak & Marschner, 1987). Low molecular weight complexes commonly take part in the greatest portion among these soluble Zn forms and are possibly the most significant form of active Zn.

Most of the soluble Zn within the cell is combined with low molecular weight anionic complexes, but only a small percent presents as free ions (Brown et al, 1993). In plant leaves, the presence of soluble Zn is mainly as an anionic compound probably combine with amino acids. Walker and Welch (1987) found that in lettuce leaf, 73% of total soluble Zn is the low molecular weight fractions and this percentage of total soluble Zn equals to 58% of total Zn in leaf. The amount of free Zn in leaf tissues is generally very low ranging from 5.8% (in tomato, Bowen et al., 1962) to 6.5% (in alfalfa, Johnson & Schrenk, 1964) of total Zn.

Free Zn activity could be controlled by the cell wall. The relationship which was shown by Turner (1970) and Turner and Marshall (1971), between the affinity of cell wall extracts for free Zn and the relative tolerance of different species to excess Zn, suggests a particular role for the cell wall in controlling free Zn in the cell.

The Zn supply to physiologically active macromolecules may be affected by the existence of significant levels of low molecular weight Zn complexes. Since the pool of low molecular weight Zn complexes would be easily degradable, it could be accepted as physiologically active (Brown et al., 1993) as in the same way that Zn incorporated in enzymes is physiologically active (Olsen, 1972).

As mentioned by Brown et al. (1993), these low molecular weight species may also play a role in Zn detoxification. Excessive metal concentrations could be absorbed by various ligands in the cell wall acts as a buffer system. One of these ligands is the phytochealtins isolated by Grill et al. (1985).

Although only a few Zn-containing enzymes such as alcohol dehydrogenase, Cu-Zn-SOD, carbonic anhydrase (CA) and RNA polymerase are identified in higher plants, a larger number of enzymes are made active by Zn, either due to binding enzymes and substrates, or effects of Zn on structure of enzymes or substrate, or both (Römheld & Marschner, 1991). The Zn atom is usually tightly connected to the apoenzyme and can be removed only with severe chemical treatments (Brown et al., 1993).

Though Zn is bounded through imidazole and cysteine by the most of enzymes, the main Zn-containing enzymes, and enzymes that needs Zn for activity are very different in their properties (Brown et al., 1993).

2.2.1.2. Functions of zinc in plants

Carbohydrate metabolism: Carbohydrate metabolism at various levels can be affected by Zn-dependent enzymes (Cakmak, 1988). When plants have an increasing Zn stress, carbonic anhydrase (CA) activity sharply declines (Ohki, 1976) and this decline is the most clear change in activity of enzymes of the carbohydrate metabolism (Shrotri et al., 1983). Localization of CA takes part normally in the cytoplasm and chloroplasts and CA may expedite the transfer of CO_2/HCO_3^- for photosynthetic CO_2 fixation (Römheld & Marschner, 1991). Parma et al. (1995) showed that in cauliflower, probably as a component of CA needed for maintaining adequate $[HCO_3^-]$ in the guard cells, and as a factor affecting K+ uptake by the guard cells, Zn involves in stomatal opening. Although there is a close relationship between Zn nutritional status and CA activity, no clear relationship between CA activity and photosynthetic CO_2 fixation exists (Römheld & Marschner, 1991). More recently, the hypothesis that CA functions to facilitate the supply of CO_2 from stomatal cavitiy to the site of CO_2 fixation has been supported by the findings in rice leaves that Zn deficiency enhanced the transfer resistance from the stomatal cavity to the site of CO_2 fixation 2.3-fold (Sasaki et al., 1998). Furthermore, Ajay and Rathore (1995) showed a close relationship between Zn nutritional status and ^{14}C-fixation in rice seedlings. Besides, Fischer and his colleagues (1997) mentioned that two wheat genotypes differing in Zn efficiency also had different gas exchange characteristics, in such a way that the Zn efficient genotype showed higher rates of the net CO_2, especially under a low Zn supply. Despite a decline in enzyme activities and in the rate of photosynthesis in most instances sugars and starch accumulate in Zn-deficient plants (Sharma et al., 1982). Marschner (1995) suggested that Zn-deficiency induced changes in carbohydrate metabolism are not primarily responsible for either growth deceleration or the occurrence of visible symptoms of Zn deficiency.

Protein synthesis: While the amino acids and amides are increased in Zn-deficient plants, the protein synthesis and the protein content in these plants are declined (Marschner, 1995). Mainly a marked decline in ribonucleic acid causes inhibition in protein synthesis in Zn-deficient plants. Either lower activity of the Zn-containing RNA polymerase (Soloiman & Wu, 1985), reduced structural integrity of ribosomes (Obata & Umebayashi, 1988), or enhanced RNA degradation (Cakmak et al., 1989) are the main reasons for this marked decline of RNA in Zn-deficient plants.

Zinc is needed for maintenance of integrity of biomembranes. It might bind to phospholipid and sulfhydryl groups of membrane constituents or form tetrahedral complexes with cysteine residues of polypeptide chains and thus protect membrane lipids and proteins against oxidative damage (Marschner, 1995). The Zn-containing isoenzyme of superoxide dismutase (Cu-Zn-SOD) has a very important role in the protection of membrane lipids and proteins against oxidation by the means of the detoxification of superoxide radicals (O_2^{-}) (Cakmak & Marschner, 1988b; Cakmak et al., 1989). In Zn-deficient plants, the activity of Cu-Zn-SOD (Marschner & Cakmak, 1989) and the catalase activity (Cakmak et al., 1989), involved in detoxification of hydrogen peroxide (H_2O_2), decrease. The high levels of toxic oxygen-free radicals in Zn-deficient plants destroy the double bounds of polyunsaturated fatty acids and phospholipids in the membranes, and enhance leakage of solutes such as K^+, sugars, and amino acids (Cakmak and Marschner, 1988a). The destruction of chlorophyll, necrosis, and reduction in plant growth, particularly under high light intensity are caused by increased lipid oxidation (Marschner & Cakmak, 1989).

The reasons for lower concentrations of phytohormones, in particular indolacetic acid (IAA), in Zn-deficient plants are either enhanced oxidative degradation of IAA as a result of increased peroxidase activity and oxygen free radicals as mentioned by Cakmak (1988), or decreased synthesis of IAA.

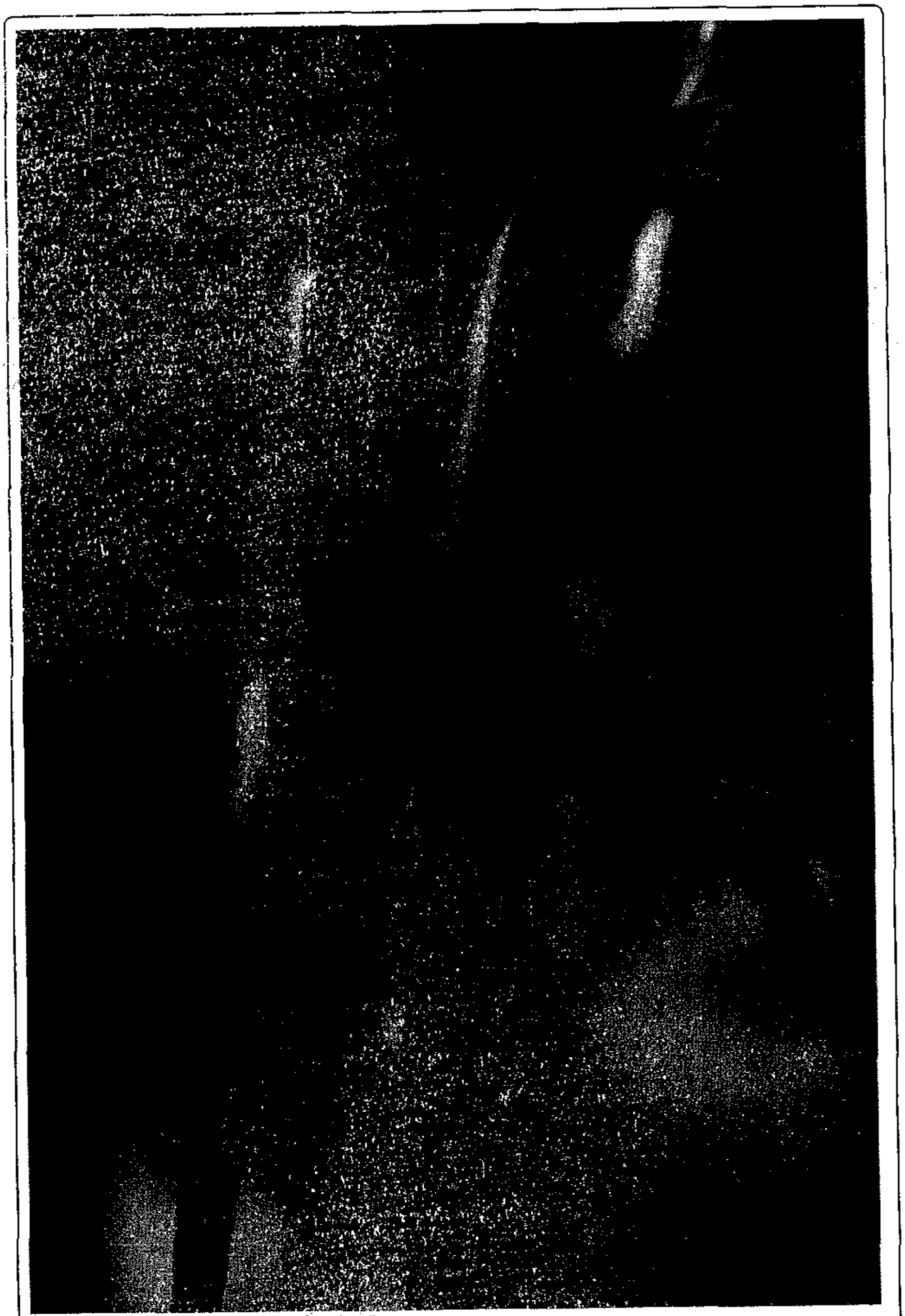
Because of its preferential binding to SH-groups, Zn plays a key role in stabilization and structural orientation of particular membrane proteins. Decrease in membrane integrity of Zn-deficient plants causes to the increase in susceptibility to fungal diseases (Sparrow & Graham, 1988), and excessive P uptake by roots and transport into the shoots, causing P toxicity in shoots (Welch et al., 1982; Marschner & Cakmak, 1986). However, in a more recent study, it has been shown that an increase in the expression of high-affinity P transporter is responsible for the excessive P uptake by the roots not the decreased membrane integrity of the Zn-deficient plants (Huang et al., 2000).

2.2.2. Critical tissue concentrations and deficiency symptoms

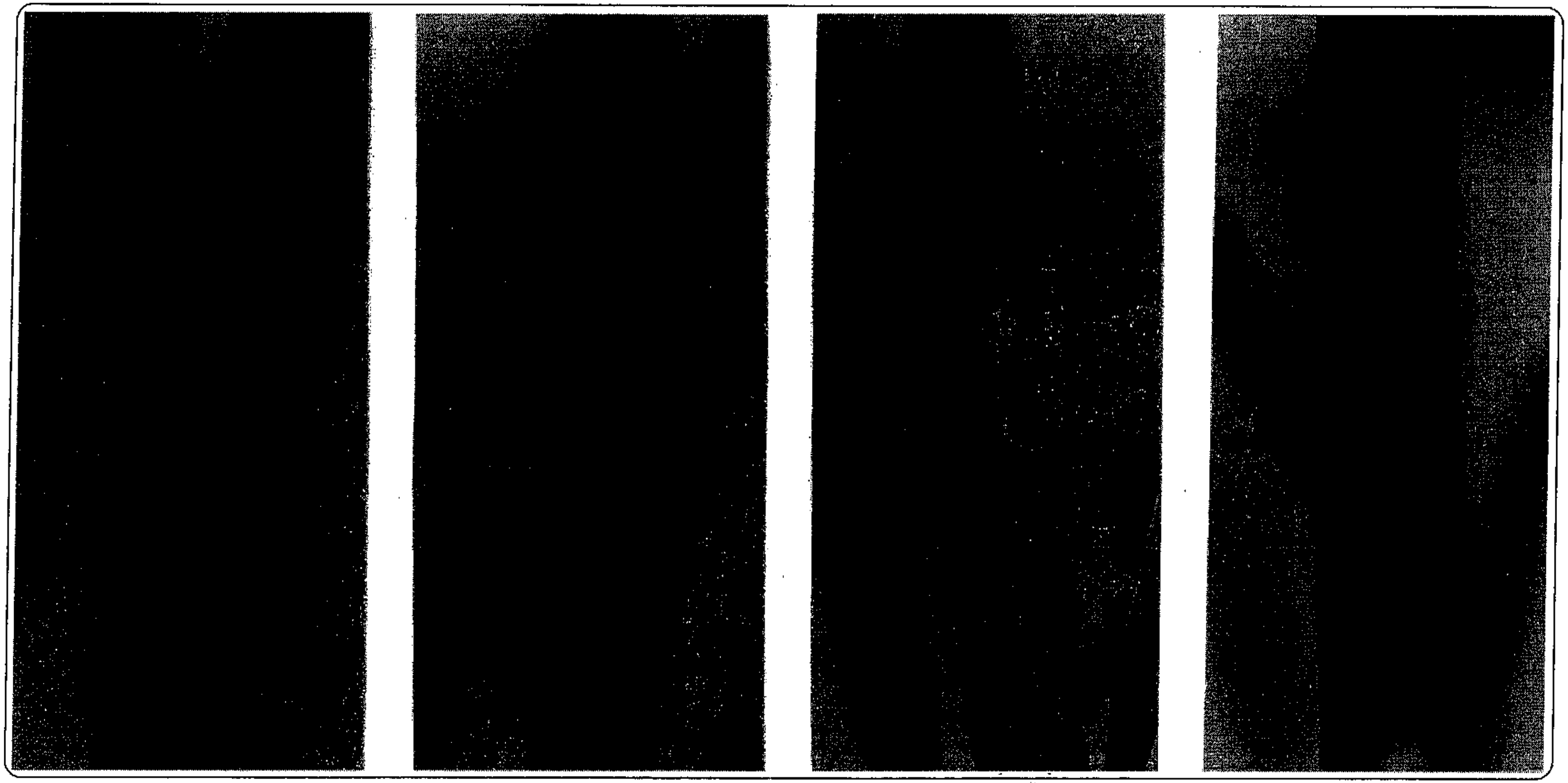
The critical deficiency concentration (CDC) of Zn in mature leaves for many crops is around 15 mg kg^{-1} which is unique since critical deficiency concentrations change significantly from one species to another one (Jones, 1991). Cakmak and Marschner (1987) and Bergmann (1993) suggested that the CDC's of Zn in leaves vary between 15 to 30 mg kg^{-1}. They depend on leaf age (Reuter et al., 1982) and may be higher with high or excessive P concentrations in the leaf tissue. In such a way that Cakmak and Marschner (1987) expressed that particularly at high P levels, instead of total Zn, water-soluble Zn seems to be a better parameter for the CDC. In each case, beside absolute Zn content, P/Zn ratios in plant tissue give very important information about the Zn nutritional status of plants (Bergmann, 1993). Depending on plant species and genotypes within a species, the values between 50-200 are accepted as optimum (Bergmann, 1993). A very small change in concentration of Zn (generally only 1 or 2 mg kg^{-1}) can mean the difference between deficiency and sufficient (Jones, 1991 and references therein).

The critical toxicity concentrations of Zn, as with most of the other micronutrients, are not well-known. For many species, either significant yield reductions and/or toxicity symptoms will occur at Zn leaf concentrations >100 mg kg^{-1} (Jones, 1991). As mentioned by Römheld and Marschner (1991), this concentration can reach values up to 8000 mg kg^{-1} in Zn-tolerant ecotypes of natural vegetation.

The rosetting and a decrease in leaf area are the well-known visible symptoms of Zn deficiency. Stunted growth and chlorosis on the youngest leaves present together (Römheld & Marschner, 1991). In monocotyledones, particularly in corn, chlorotic bands appear along the central veins of leaves combined with red, spot-like stain. In the case of wheat, after the reduction in shoot elongation, the development of whitish-brown necrotic patches on the middle parts of leaves, reddish-brown patches for barley, is characteristic (Cakmak et al. 1998). These typical whitish-brown necrotic patches and the developmental stages of them are given in Picture 2.1 and 2.2.



Picture 2.1. Zinc deficiency symptoms in wheat grown in field



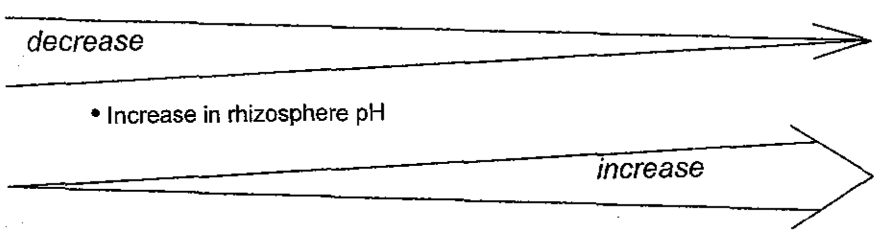
Picture 2.2. Development of Zn deficiency symptoms, whitish-brown necrotic patches on middle part of leaves, in wheat grown on a Zn deficient soil in Central Anatolia, Turkey

2.2.3. Mechanisms of zinc uptake and translocation in plants

2.2.3.1. Zinc uptake

A. Role of rhizosphere changes in concentrations and dynamics of zinc

Rhizosphere soil differs in many instances than bulk soil as for example in pH, the presence of root exudates, and microbial activity. Most of the root-induced changes caused by P or Fe deficiency are also of particular importance to the dynamics and mobilization of Zn in the rhizosphere. Main plant factors decreasing of increasing Zn availability and uptake by soil grown plants are schematized in Figure 2.3 adapted from Marschner (1993).



- Decrease in rhizosphere pH
  - form of nitrogen supply
  - excretion of organic acids
- nutrient deficiency-induced changes
  - phosphorus deficiency (root surface ares, excretion of H^+ and organic acids)
  - iron deficiency (acidification, release of chelators)
  - zinc deficiency (acidification, release of chelators)

Figure 2.3. Main plant factors decreasing or increasing zinc availability and uptake by soil grown plants (Marschner, 1993)

Root induced changes in pH

The pH values of rhizosphere and bulk soil may differ from each other up to two units and affected by plant and soil factors. Imbalance in cation/anion uptake ratio and the corresponding differences in net release of H+ and HCO_3^- (or OH^-), secretion of organic acids, and increased CO_2 production are the most important factors which are effective on root-induced changes in rhizosphere pH (Marschner, 1993). Organic acids may also be produced by microbial activity stimulated by the release of organic carbon from roots, and CO_2 production by roots and rhizosphere microorganisms.

The extend to which plant roots can change the rhizosphere pH is mainly affected by the factors, the pH buffering capacity of the soil and the initial pH (bulk soil) (Marschner, 1993). Cation/anion uptake ratio, and as a result of this, the rhizosphere pH are most noticeably affected by the form of nitrogen supply (Marschner & Römheld, 1983). In neutral and alkaline soils, rhizosphere acidification in plants supplied with ammonium can enhance mobilization and uptake of micronutrients such as Zn, as well as of P. The Zn uptake rates per unit root length increased from 7 $\mu g\ m^{-1}$ in nitrate fed bean plants to 13 $\mu g\ m^{-1}$ in ammonium fed plants (Thomson et al., 1993).

The concentration and mobility of Zn in the soil solution are decreased with the increase in rhizosphere pH in plants supplied with nitrate (Marschner, 1993).

The effect of NH_4 nutrition on acidification of the rhizosphere increases the availability of Zn for plants growing in soil with high pH of bulk soil and in soils with low extractable Zn. In soils with high contents of the extractable Zn, however, plant growth can be depressed by Zn toxicity, because of induced Zn uptake in excess of Zn demand by ammonium supply.

Plant-borne organic acids and chelators (root exudates)

The term of *root exudates* covers both high and low-molecular-weight solutes released or secreted by the roots. The most important high-molecular-weight solutes are musilage and ectoenzymes and those of the low-molecular-weight fraction are organic acids, sugars, phenolics, and amino acids (Marschner, 1995). Numbers of endogenous and exogenous factors play an important role in root exudation (Uren & Reisenauer, 1988). Among them, the nutritional status of plants and the mechanical impedance of the substrate seem to have particular importance for nutrient acquisition and nutrient dynamics in the rhizosphere (Marschner, 1995).

Large amounts of citric acid secreted by *Lupinus albus,* causes to exact decrease in the rhizosphere pH in the zones of root clusters, the so-called proteoid roots, even in soils with high $CaCO_3$ content (Dinkelaker et al., 1989). This mechanism leads to mobilize the sparingly soluble calcium phosphates. According to Marschner (1993), as a side-effect, this decline in the rhizosphere pH causes also the increase in the mobilization of Zn, Mn, and Fe in the rhizosphere soil.

As typical root responses to Fe deficiency, dicots and monocots (with the exception of Graminacea) increase the rhizosphere acidification by an increase in net proton excretion, together with excitation of reducing capacity of the roots, and generally associated with enhanced release of phenolics from the roots (Römheld, 1987). By this root response, not only Fe mobilization in the rhizosphere and uptake by plants, also of Mn is enhanced (Marschner et al., 1986). It was suggested that the Zn mobilization in the rhizosphere and uptake by plants could also be increased by the root responses to Fe deficiency and this case possibly more common on calcareous soils than considered (Marschner, 1993).

Graminaceous species respond to deficiencies of Fe (Römheld, 1987) and Zn (Cakmak et al., 1994; Zhang et al., 1989) by an enhanced release of non-proteinogenic amino acids, so-called phytosiderophores (PS's). Phytoiderophores are highly effective on complexing and mobilizing Fe and Zn in the rhizosphere (Takagi et al., 1988; Treeby et al., 1989) and within the root apoplast (Zhang et al., 1991) are as effective as DTPA in mobilizing Zn from calcareous soils (Treeby et al., 1989). The possible role of PS's in Zn uptake will be discussed as more detailed later on (see *page* 33).

By numbers of root-induced changes in the rhizosphere, plants also respond to Zn deficiency, for example even when the plants supplied with nitrate, the rhizosphere pH declines in Zn deficient dicots is attributed to an increase in the cation/anion uptake ratios (Cakmak & Marschner, 1990). Beside this, the efflux of low-molecular-weight organic solutes such as sugars, amino acids, and phenolic is enhanced as a result of increased permeability of membranes in the root cells (Cakmak & Marscher, 1988). However, as shown by Marschner and his colleagues (1990), the enhanced mobilization of Zn from Zn loaded resin occurs only in the presence of the root exudates of the graminaceous species, not of the dicots.

Besides the direct role of root-induced changes in solubilization and mobilization of Zn in the rhizosphere, these properties of Zn can indirectly be affected by the presence of non-infecting rhizosphere microorganisms, for example by the production of chelators (Marschner, 1993).

Arbuscular mycorrhizae (AM)

The most distinct growth enhancement effect of AM occurs by improved supply of mineral nutrients with low mobility in the soil, in particular phosphorus (P). Phosphorus can be absorbed and transferred to the host from soil outside the root depletion by the external hyphae zone of nonmycorrhizal roots (Tinker et al., 1992). The micronutrients, Zn and Cu, may also be transported to the host by the same way (Marschner, 1993). The effect of AM on Zn acquisition by the host is generally more distinct in soils low in extractable Zn, or low in mobility and in plants with coarse root systems, for example fruit trees such as peaches (Gilmore, 1971), citrus (Menge et al., 1982), and apple (Runjin, 1989). Zinc uptake and concentrations are also enhanced in graminaceous species such as maize and wheat grown in soils with low Zn contents by AM (Swamvinathan & Verma, 1979; Faber et al., 1990). This role of AM is not only limited with the results of pot experiments, but for barley such an effect was also shown in field conditions (Jakobsen, 1983).

Zinc can absorbed and translocated, *similar to* P, to the host from outside of the rhizocylinder by external hyphae of AM. According to Marschner (1993 and references therein), 50-60% and 25% of the total Zn uptake, respectively in white clover and in maize may be delivered by the external hyphae in an experiment conducted by using .

When the plants are supplied with P, the amounts of Zn provided through AM hyphae decrease, causing limitations for the Zn nutrition of plants. As shown in soybean (Lambert & Weidensaul, 1991) and maize (Eiwazi & Weir, 1989), when the plants grown on a P-deficient soil supplied with P, growth is generally sharply increased as well as the P concentration in the shoot, but Zn concentration decreases. However, in plants with AM, even the growth is increased, both P and Zn concentrations enhance, showing the importance of AM in delivery of Zn and P to the host plant.

The plants supplied with P or grown in the soils with high P contents, generally have low root colonization rates of AM (Singh et al., 1986). In view of Marschner (1993), while P demand of the host can be provided by higher root uptake due to elevated soil solution concentrations and diffusion rates, for Zn such a compensation does not happen. Such depressions in mycorrhizal activity in roots can often cause an increase in Zn concentrations in the shoots which exceed values that is *explainable* by dilution on soils with low Zn availability (Singh et al., 1986). This negative effect of reduced mycorrhizal activity may be eliminated by using sparingly soluble phosphate fertilizers and phosphate fertilizers which contains Zn (Pairunan et al., 1980; Brennan & Gartrell, 1990).

Many results published in recent years show that low root colonization by AM is generally responsible for low Zn contents in host plants with AM.

However, there are also some studies in which such a role of AM does not exist (Lu & Miller, 1989; Weber et al., 1993).

B. Zinc absorption into roots

Zinc is an essential micronutrient that goes into plants primarily via absorption of Zn^{2+} from the soil solution by the roots. There are relatively few studies in the literature dealing with the mechanism(s) and regulation of Zn^{2+} absorption by plant roots comparing to Fe. Almost all of these studies have been conducted using solution culture techniques. Two different nutrient solution types are used in studies related with Zn. One of them is "conventional hydrophonic solution" in which Zn^{2+} concentrations which support sufficient plant growth range from 0.05 to 0.25 μM (Carroll & Loneragan, 1968). "Chelator-buffered hydrophonic solution" is the second one and more recent studies based on this technique (Chaney, 1989; Norvell & Welch, 1993; Rengel & Graham, 1995b, 1996). Controlling free Zn^{2+} activity at especially suitable low activities is the most important advantage of this technique. The results of the experiments conducted by using chelator-buffered solution technique brought to light that actually, the critical Zn^{2+} activity in the rhizosphere is much lower in the range of 25-160 pM for tomato and barley (Chaney et al., 1989; Norvell & Welch, 1993).

Solution culture techniques used in studies of Zn^{2+} absorption

Three different approaches (techniques) have been operated in the studies of Zn^{2+} absorption (Kochian, 1993). The first one is use of the radioisotope, $^{65}Zn^{2+}$, in short-term uptake experiments to quantify unidirectional Zn^{2+} influx. In addition, roots can be kept longer in $^{65}Zn^{2+}$ containing solution until tracer flux equilibrium is achieved. The second one can only be operative if the ratio of root/uptake solution is attentively managed. In this case, for rapid depletion of Zn^{2+} from solution conditions would be set up. This change in extend Zn^{2+} concentration may be observed, as a result of all these steps, net Zn^{2+} uptake rates can be calculated. By using a solution containing a constant Zn^{2+} level, such as chelator-buffered nutrient solution, long-term Zn^{2+} absorption rates can be determined (the third approach).

Kinetics of zinc uptake

Either the time-dependent or concentration-dependent kinetics of Zn^{2+} uptake have been used intensively by the researchers who were interested in root Zn^{2+} absorption. The results of the studies done as a function of time to characterize Zn^{2+} accumulation in plant roots show the existence of a biphasic uptake. At the beginning, Zn enters rapidly and is bounded within the root-cell-wall and is followed by a slower, linear phase of transport across the plasma membrane (Kochian, 1993). The initial rapid phase can be easily desorbed with other divalent cations or non-labelled Zn^{2+} solutions. Shimid et al. (1965) studied $^{65}Zn^{2+}$ uptake from either 1 or 5 mmol m^{-3} $ZnCl_2$ solution in excised roots. They observed a linear uptake over the first 2h, and most component (60%) of this phase was diffused into and bounded in the apoplasm, and the rest was taken up due to a plasma membrane transport-mediated process.

Using an application on an enzyme kinetic analysis almost the same to root mineral ion absorption which was used firstly by Epstein and Hagen (1952), concentration dependency of Zn^{2+} have been tested in number of experiments. The conclusions of this approach are that, saturating transport is made easier by an enzyme-like transport protein with specific transport characteristics and Michaelis-Menten kinetics (K_m and V_{max}) can be used to indicate this saturating transport. When concentration-dependent kinetics of Zn^{2+} uptake in plants are considered, it can be easily expressed that saturation kinetics are often observed. But the K_m values obtained in these studies show conflicting results. For example, in some of them, K_m values are quite high, ~50 mmol m^{-3} (Bowen, 1981, 1986; Ramani & Kannan, 1978), although it is known that the concentration of Zn^{2+} accepted as adequate is well below 1 mmol m^{-3}. In a study conducted by using intact maize roots, kinetic parameters of Zn^{2+} uptake were determined, using a more realistic concentration range, 0-1.5 mmol m^{-3} (Mullins & Sommers, 1986). The obtained K_m value for uptake of between 1.5 and 2.2 mmol m^{-3} seems to be more realistic for field-grown plants.

Compartmental analysis technique was applied in the roots of wheat by Santa Maria and Cogliatti (1988). Estimations on compartmentation was done from "wash-out" experiment. According to the obtained results from this experiment, they mentioned that there is a fast exchanging compartment, presumably located in the free space, equal to 16% of the total root Zn content; a slower exchanging compartment, suggested to be cytoplasm and accounted for about 8% of the total root content; the third and slowest component contains 76% of the total root Zn. Because of some irregular kinetic aspects of this compartment, it is suggested that this phase can be the result of more that one chemical or physical compartment. Finally, they made a suggestion that organic forms of Zn in the vacuole or cytoplasm could be present in addition to Zn^{2+}.

Zinc transport across the plasma membrane might occur via a non-specific divalent cation channel with the driving force due to primarily to the negative membrane potential (Kochian, 1993). There are some contradictory results in the literature relating the coupling of Zn^{2+} uptake to metabolism. A number of reports in algae, barley, and bean plants show that because of a lack of response to metabolic inhibitors, Zn^{2+} absorption should not be metabolically-dependent (Broda et al., 1964; Gutknecht, 1961, 1963; Rathore et al., 1970). However, the others had an evidence in support of a metabolically controlled transport of Zn^{2+} (Bowen, 1969; Giordano et al., 1974).

Transport interactions with other ions

In terms of nutrient interactions, the effect of all alkaline earth cations (Ca^{2+}, Mg^{2+}, Sr^{2+}, and Ba^{2+}) on inhibition of Zn^{2+} uptake in the roots of wheat was shown by Chaudry and Loneragan (1972). The results of kinetic analysis suggested that these ions and Zn^{2+} are not taken up via the same transport mechanism, showing non-competitively inhibited Zn^{2+} uptake. While Mn had no effect, a strong inhibition of Cu^{2+} in Zn^{2+} uptake is observed by Schimid et al. (1965). Since Bowen (1969) found a competitively inhibited Zn^{2+} uptake by Cu^{2+}, suggested that the same transport system is shared by Zn^{2+} and Cu^{2+}.

Zinc uptake in graminaceae

As mentioned before, in grasses Fe (Römheld, 1987; Takagi, 1976) and Zn (Cakmak et al., 1994; Zhang et al., 1989) deficiencies increase the synthesis and release of non-protein amino acids, called phytosiderophores (PS's), into rhizosphere. These compounds can effectively chelate, both Zn^{2+}, Fe^{3+} and Cu^{2+} (Treeby et al., 19899 and transport them to the outer face of the root-cell plasma membrane. The Fe(III)-PS (Marschner et al., 1989; Römheld & Marschner, 1986) and Zn(II)-PS (Crowley et al., 1991; von Wiren et al., 1996) are transported into the cell via a transport system.

2.2.3.2. Long-distance zinc translocation

A. Zinc transport in xylem

Zinc absorbed by the roots is rapidly translocated to the shoots (Riceman & Jones, 1958). It was found by Reay (1987) that the flux of Zn was 5 times higher than that of silicon (Si), using the ratio of different elements to Si as a measure of passive transport in *L. albus* shoot. These results show that the transport of Zn to shoots is not clearly via passive transport in the transpiration stream. As mentioned in Kochian's review chapter (1993), Zn accumulation in expanding leaves in rice, but not in fully expanded leaves which had the highest rates of transpiration, was shown by Obata and Kitagishi (1980) using ^{65}Zn autoradiography.

Zinc concentration in xylem sap is 2-6 mmol m^{-3} in stem sap of soybean and tomato with adequate Zn supply (0.5 mmol m^{-3} Zn) and 15-85 mmol m^{-3} in plants with higher Zn supply (3-8 mmol m^{-3} Zn) (White et al., 1981a). In terms of the forms of Zn in the xylem, it is still unclear. According to the results of electrophoresis of ^{65}Zn in xylem sap, Tiffin (1967) concluded a cationic transport in xylem sap. Later on, theoretical work of White and his colleagues (1981a,b) done with the CHELATE computer program implied that there is significant complexation with citric acid and malic acid in soybean and tomato. Two computer programs, CHELATE and GEOCHEM were compared for predictions of Zn complexation in xylem and phloem sap by Mullins et al. (1986). Although the theoretical basis of both programs are similar, they differ significantly in predicting on the form of Zn in xylem sap. These large differences restrict to predict the proportions in good order.

B. Zinc transport in phloem

To our knowledge the retranslocation of Zn in plants is variable and depends on the Zn supply to the plant. Marschner (1995) has put the Zn into the group of intermediately phloem mobile nutrients. According to Longnecker and Robson (1993) Zn movement out of senescing leaves of plants with adequate Zn supply is greater than with deficient supply. There is conflicting results about the retranslocation of Zn to other tissues when Zn is applied with foliar application technique. For example, Orphanos (1975) did not observe any movement of Zn from the sprayed leaves to the apices. However, other studies, most of them conducted in recent years, showed the non-limited mobility of Zn in wheat (Herren & Feller, 1994; 1997), bean (Bukovac & Wittwer, 1957; Ferrandon & Chamel, 1988), citrus (Wallihan & Heymann-Herschberg, 1956) and pistachio (Zhang & Brown, 1999).

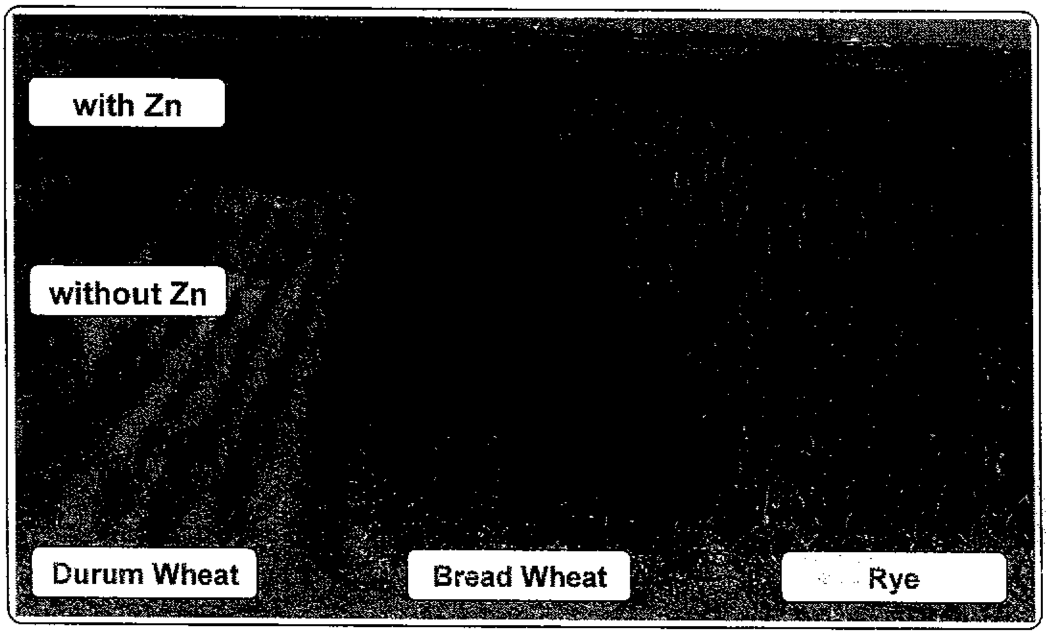
The concentration of Zn in the phloem sap range from 3 to 170 mmol m^{-3} (Robson & Pitman, 1983). The most of the Zn in the phloem sap of *Ricinus communis* was found in a negative form with two peaks distinct from the reference $^{65}Zn^{2+}$ peak by Van Goor and Wiersma (1976) using paper electrophrosis of the phloem exudate.

2.2.4. Evidence for genotypical variations

As mentioned by Graham and Rengel (1993), crop species differ in susceptibility to Zn deficiency. Oilseeds and cereals are more resistant to Zn deficiency than lentil, chickpea, and pea (Tiwari & Dwivedi, 1990). However, Graham and Rengel (1993) reminded that such comparisons should be done with very attention because of the dependency of the apparent ranking on the choice of cultivars to represent a particular species, since there are well-known differences in susceptibility to Zn deficiency among genotypes within a species.

Differential tolerance to Zn deficiency among various genotypes has been documented for a number of crops: potato (Sharma & Grewal, 1990), navy bean (Jolly & Brown, 1991), tomato (Parker et al., 1992), pearl millet (Takkar et al., 1988), sorghum (Shukla et al., 1973; Ramani & Kannan, 1985), maize (Ramani & Kannan, 1985), oats (Brown & McDaniel, 1978), and wheat (Shukla & Raj, 1974; Graham, 1991; Graham et al., 1992; Cakmak et al., 1996a, 1998).

According to Graham and Rengel (1993), large differences can be demonstrated among the following cereals: ryes > triticales > bread wheats > durum wheats. Similar to this, the results of a study done by using 6 cereal species showed that the susceptibility of these species to Zn deficiency grown on a Zn-deficient calcareous soil from Turkey declines in the order durum wheats > oats ≥ bread wheats > barleys > triticales > ryes (Cakmak et al., 1998). The differences in Zn efficiency of rye, bread wheat, and durum wheat species can be seen more clearly in *Picture* 2.3. from a field experiment conducted on a Zn deficient soil in Central Anatolia, Turkey (Cakmak et al., 1997).



Picture 2.3. Growth of durum wheat, bread wheat, and rye genotypes on a Zn deficient soil with (23 kg Zn ha^{-1} as $ZnSO_4.H_2O$) or without Zn application (Cakmak et al., 1997)

Soil type plays an important role in Zn efficiency among the genotypes of a species, for example durum wheats (Graham & Rengel, 1993). They introduced that different mechanisms involve in handling simple deficiency in deep sands and complex Zn deficiency in vertisols.

2.2.5. Possible mechanisms effective on zinc efficiency

It seems that the Zn-efficient plants improve some physiological mechanisms that allow them to resist the Zn deficiency stress better than the Zn-inefficient plants. In recent years, studies on clarifying the exact role of these mechanisms in differential Zn efficiency of genotypes, particularly in cereals, attracted the interests of the researchers.

Root growth: Differential root morphology was discussed in Rengel's review chapter (1999) and a greater proportion and longer length of fine roots with diameters ≤0.2 mm in early growth stage are mentioned as two characters associated with the Zn-efficient genotypes (Dong et al., 1995), thus resulting in touch of a larger volume to soil. As Zn ions are moved toward roots by diffusion (Warncke & Barber, 1972), an enhanced root surface area is especially important.

As discussed before, AM can increase the capacity of plant to take up Zn from soils low in available Zn. The Zn concentrations in roots of the plants, green gram (Sharma & Srivastava, 1991), maize (Faber et al., 1990; Kothari et al., 1991, Sharma et al., 1992), and pigeon pea (Wellings et al. 1991) whose roots were infected with AM, were higher than in non-mycorrhizal plants. However, in the case of wheat grown on Zn-deficient soils of Australia, Rengel (1999) mentions that the role of AM in Zn uptake is negligible. This negligible role of mycorrhizae is attributed to low level of natural mycorrhizal inoculum (Graham & Rengel, 1993) and nil mycorrhizal infection rate in the soil with P supply.

Chemistry and biology of rhizosphere: Qualitative and/or quantitative differences in root exudation among species and cultivars of a species could be responsible for differential nutrient efficiency. In the same way, under Zn deficiency, Zn-efficient cereal genotypes release greater amounts of PS's (Cakmak et al., 1994; 1996c; Rengel et al., 1998; Walter et al., 1994).

Preliminary results propose the possible existence of differentially affected microbial populations in the rhizosphere of wheat genotypes differing in Zn efficiency (Rengel et al., 1996; Rengel, 1997b; Rengel et al., 1998). But on this topic additional studies are needed.

Zinc uptake and transport: When wheat genotypes were compared for their net Zn uptake rates from chelator-buffered nutrient solution, it was found that there were genotypical differences (Cakmak et al., 1998; Rengel & Graham, 1995b, 1996; Rengel & Hawkesford, 1997; Rengel et al., 1998; Rengel

& Wheal, 1997). However, Rengel and Graham (1995b) could not see any significant correlation between the rate of net Zn uptake and dry matter production, in the study done by using large number of genotypes under low Zn supply. When this comparison was done for the most Zn-efficient and most Zn-inefficient wheat genotypes, a significant relation appeared (Rengel & Graham, 1996; Rengel & Hawkesford, 1997; Rengel et al., 1998; Rengel & Wheal, 1997b). However in a study in which one Zn-efficient and one Zn-inefficient bread wheat and one Zn-inefficient durum wheat genotypes were tested, no increased Zn uptake rate by Zn-efficient genotype could be detected (Wheal & Rengel, 1997). Cakmak et al. (1998) using rye, bread wheat and durum wheat genotypes conducted an short-term uptake experiments in chelator-buffered nutrient solution and according to the output of the study, concluded that there is a well-accordance between net Zn uptake rates and Zn efficiencies of genotypes used. In such a way that the most Zn-efficient rye and the most Zn-inefficient durum wheat have the highest and lowest uptake rates, respectively (Cakmak et al., 1998). Bread wheat cultivars used in that work also tended to differ from each other corresponding to their susceptibility to Zn deficiency.

Higher Zn translocation from roots to shoot was shown in Zn-efficient wheat genotypes under Zn deficiency than in Zn-inefficient plants (Cakmak et al., 1996b; Rengel et al., 1998).

Utilization of zinc in roots: Total Zn concentration in the roots and the shoot is not a good parameter to express the Zn nutritional status of plants, especially with low Zn supply (Cakmak et al., 1996a,b, 1998; Rengel & Graham, 1995b). Dong et al. (1995) could not find any difference in total Zn concentration in roots and shoots among wheat genotypes differing in Zn efficiency when grown under Zn-deficient conditions in soil which was an plant growth medium named as sandy soil but actually it could be called directly as a sand culture. The sand culture have more similarities to the nutrient solution culture than to the soil culture. In the same way, root concentrations measured by Dong et al. (1995) of plants were similar to the root concentrations of plants grown under Zn deficiency in nutrient solution culture (Cakmak et al., 1996b; Erenoglu et al., 1996, 1999; Rengel and Graham, 1995b).

Although the same root and leaf concentrations of total Zn in the Zn-efficient and the Zn-inefficient genotypes, only Zn-inefficient genotypes showed the necrotic patches on leaf blade which is one of the typical visual symptoms of Zn deficiency (Cakmak et al., 1994; 1997a,b; Rengel & Graham, 1995a; Rengel & Hawkesford, 1997). The Zn-efficient genotypes also had significantly more shoot dry matter than the Zn-inefficient genotypes, although there was no difference between the two groups in the total Zn concentration in shoot. Because of this reason, the greater internal utilization capacity is discussed as a possible reason of genotypical differences in Zn efficiency.

The higher activities of carbonic anhydrase (CA) measured in the Zn-efficient genotypes than in the Zn-inefficient genotypes may be also a reason for genotypical differences in Zn efficiency (Rengel, 1995). According to Rengel (1999), the higher activity of CA in the Zn-efficient bread wheat genotype compared to the Zn-inefficient durum wheat genotype under Zn deficiency stress seems to be an consequence, rather than a cause, of differential Zn efficiency because of apperance of differential decrease in growth under Zn deficiency before significant differences in CA activity between the genotypes were measured (Rengel, 1995).

As seen in a whole range of other environmental stresses, an increased production of free oxygen radicals occurs also in Zn deficient plants (Cakmak & Marschner, 1988b). And the Cu-Zn-SOD plays a very important role in the protection of membrane lipids and proteins against oxidation by the means of the detoxification of O_2^{-}(Cakmak & Marschner, 1988b; Cakmak et al., 1989). In the same way that Cakmak and his colleagues (1997) found a higher Cu-Zn-SOD activity in Zn-efficient bread wheats and rye than in the Zn-inefficient durum wheat.

Zinc-deficient barley (Welch and Norvel, 1993) and wheat (Rengel, 1995b) plants had less amounts of 5,5'-dithio-bis(2-nitrobenzoic acid)-reactive sulfhydryl groups than Zn-sufficient plants. In the same way that Rengel (1995b) found a greater amount of sulfhydryl groups in Zn-efficient than Zn-inefficient one, without making any allowance for Zn supply (Rengel, 1995b).

Biosynthesis of polypeptides in the root-cell plasma membranes of wheat genotypes with different Zn efficiency is affected differentially by various Zn supplies (Rengel & Hawkesford, 1997). In this work, it was suggested that the polypeptide could play a role in Zn efficiency relying on increased biosynthesis of the 34-kDa polypepdide under Zn deficiency in the root cell plasma membranes of the Zn-efficient bread wheat genotype, but not in the Zn-inefficient durum wheat genotype. However, as mentioned by Rengel (1999) they could not find again a well-accordance between biosynthesis of the 34-kDa polypeptide and Zn efficiency of a number of wheat and barley genotypes grown under Zn deficiency.

3. Different responses of cereals (rye, bread wheat, and durum wheat) to zinc deficiency on calcareous soils

3.1. Abstract

A greenhouse experiment was carried out to study the responses of rye (*Secale cereale* L. cv. Aslim), two bread wheats (*Triticum aestivum* L. cvs. Bezostaja and BDME-10), and durum wheat (*Triticum durum* L. cv. Kunduru-1149) cultivars to zinc (Zn) deficiency and Zn fertilization in a calcareous soil with extremely low available Zn (DTPA extractable Zn= 0.09 mg kg^{-1} soil). The first visible symptom of Zn deficiency was reduction in shoot elongation followed by the appearance of whitish-brown necrotic patches on the leaf blades of older leaves. These necrotic patches occurred more rapidly and severely in wheat cultivars, particularly in the durum wheat cv. Kunduru-1149, and later on, in the bread wheat cv. BDME-10. The bread wheat cv. Bezostaja was affected less by Zn deficiency compared to the other wheat cultivars. In the rye cv. Aslim, such symptoms were not observed. The genotypes showed a similar trend to the decrease in shoot dry matter production as to the appearance time of Zn deficiency. For example, after 30 days of growth in soil, Zn efficiency of cereals, expressed as the ratio of shoot dry matter produced under Zn deficiency compared to the treatment with Zn fertilization were 77% for rye, 58% for the Zn efficient bread wheat cv. Bezostaja, 49% for the Zn inefficient bread wheat cv. BDME-10 and 47% for durum wheat. Between differences among and within the cereal species in susceptibility to Zn deficiency and the total amount of Zn per shoot (content), clear relations were found. This close correlation also existed between P/Zn ratios and Zn efficiency values of genotypes under Zn deficient condition. However, the tissue concentration of Zn was not a good

indicator for differential Zn efficiency of cereals. The results show an exceptionally high Zn efficiency of rye and there are very distinct differences in response to Zn deficiency in calcareous soils among the cereals species. These differences are presumably related to a greater Zn acquisition of efficient genotypes from the soil compared to inefficient genotypes.

3.2. Introduction

Soils with low plant-available Zn are common all over the world, particularly in calcareous soils of arid and semi-arid regions. In a global study conducted by Sillanpää (1982) in the name of FAO, it was demonstrated that approximately 30% of cultivated soils of the world is Zn deficient. Zinc deficiency possibly the most widespread micronutrient deficiency in cereals (Graham et al., 1992), especially in wheats, limiting grain yield, for example in Australia, and India (Takkar & Brennan, 1993). Not only grain yield of plants is reduced by Zn deficiency, also nutritional quality of grains (Cakmak et al., 1998). As in soils and plants, humans, particularly in developing countries, are also in danger of Zn deficiency. High consumption of cereal-based foods with low levels and poor bioavailability of Zn is discussed as a major causal factor for widespread occurrence of Zn deficiency in humans (Graham & Welch, 1996).

Also in Turkey, Zn deficiency is an important nutritional problem in plants. The results of analysis of 1511 soil samples collected from different regions of Turkey showed that approximately 50% of cultivated soils is low in plant-available Zn (Eyüpoglu et al., 1994). High pH, $CaCO_3$, and heavy texture and low levels of organic matter and soil moisture were presented as major factors lowering Zn availability to the roots in Central Anatolia region in Turkey (Cakmak et al., 1996a). Decrease in grain yield of wheat which varies between 5-554%, was reported on soils with extremely low plant available Zn in semi-arid regions of Turkey (Cakmak et al., 1996a).

The use of Zn fertilizers to eliminate the problem of Zn deficiency is a common application. However, plant species (Moraghan, 1984) and cultivars within a species, particularly wheat (Cakmak et al., 1996b, 1998; Graham et al., 1992) greatly 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. Cakmak and his colleagues (1998) reported that susceptibility of cereals to Zn deficiency grown on a Zn-deficient calcareous soil decreases in the order durum wheat > oat ≥ bread wheat > barley > triticale > rye.

Tolerance to soils with low Zn availability, as a genetic trait, is termed *Zn efficiency* and characterized as the ability of a genotype (species etc.) to grow and yield well in Zn-deficient soils for a standard genotype (Graham, 1984). Zn efficiency -determined by calculating the ratio of yield in -Zn treatment to yield in +Zn treatment and expressed in percentage- is used for characterization of the genotypic variations among plants to tolerate Zn deficiency (Cakmak et al., 1996b, 1998; Graham et al., 1992; Khan et al., 1998).

According to Shukla and Ray (1974), the genotypical differences in wheat for Zn efficiency could be related to differences in acquisition of Zn from soil. Studies with different species and wheat genotypes grown in soil showed that the concentration of Zn in shoot tissue is not a suitable parameter for evaluation of genotypical differences in Zn efficiency (Graham et al., 1992; Cakmak et al., 1996b, 1998). In contrast, the total amount of Zn per shoot was introduced as a better indicator for the differential sensitivity of cereals in Zn deficient growth medium (Cakmak et al., 1998).

The topic of the interaction between Zn and P has been discussed very often as reviewed by Loneragan and Webb (1993). But it is still a matter of discussion together with so many confusing results. One of the discussion points is the P/Zn ratio and its role in the appearance of Zn deficiency symptoms. For example, in grape leaves it was found that critical values of P/Zn are around 150 for plants grown in soil (Marschner & Schropp, 1977). In addition, as Loneragan and Webb (1993) expressed, the well-correlation between leaf symptoms and as well leaf P concentrations as the ratio of concentrations P/Zn (Webb, 1987) leading to examination of the relation Zn deficiency and P concentrations in plants. Plants normally control their capacity to take up P to maintain the P concentration in shoot tissue within the physiological limits. They decrease the capacity of the P uptake when grown under high-P conditions but increase this capacity when grown under low-P conditions (Clarkson & Scattergoor, 1982; Jungk et al., 1990). However, when exposed to low levels of Zn, high amounts of P are accumulated in the tissues of both dicots and monocots (Welch et al., 1982; Cakmak & Marschner, 1986; Webb & Loneragan, 1988; Welch & Norvell, 1993). This suggests that Zn-deficient plants loose control over the absorption mechanism of P (Safaya & Gupta, 1979; Marschner & Cakmak, 1986). Recently, it has been shown that Zn deficiency increases the expression of high-affinity P transporter in barley roots (Huang et al., 2000).

The objective of this study was to compare the responses of one rye, two bread wheat, and one durum wheat genotypes which would be used in forward parts of this PhD work, in the severely Zn-deficient calcareous soil from Central Anatolia region in Turkey.

3.3. Materials and Methods

One rye (*Secale cereale* L. cv. Aslim), two bread wheat (*Triticum aestivum* L. cvs. Bezostaja and BDME-10) and one durum wheat (*Triticum durum* L. cv. Kunduru-1149) were used in the experiment carried out under greenhouse condition. The effect of Zn supply on shoot dry matter production and concentration and content of Zn in shoots was studied using a soil extremely low available Zn from Eskisehir-Central Anatolia with. Some physical and chemical properties of this soil is given in *Table 3.1.*

Table 3.1. Some of the chemical and physical properties of the soil from Central Anatolia region in Turkey used in the experiment

pH (1:1)	Salt (%)	Organic Matter (%)	$CaCO_3$ (%)	Texture Class
8.04	0.08	0.69	14.9	Clayey (C)

DTPA-Extractable Micronutrients ($mg\ kg^{-1}$ soil)			
Zn	Fe	Mn	Cu
0.09	2.64	2.13	1.05

The experiment was carried out with 650 g of soil in a plastic pot. Two levels of Zn, 0 and 5 mg Zn kg^{-1} soil as $ZnSO_4.7H_2O$, were added together with a basal treatment of 200 mg N kg^{-1} as $Ca(NO_3)_2$, 100 mg P kg^{-1} soil as KH_2PO_4 and 2 μg FeEDTA g^{-1} soil. All nutrients were mixed throughly with the soil before sowing. Each treatment had four replicates. About 8 seeds were sown; after germination the seedlings were thinned to 4 seedlings per pot. All pots were randomized every two days and watered daily to about field capacity using deionized water. Plants were harvested 23 and 30 days after planting. Only shoots were harvested, thereafter dry weights were determined after drying at 70 °C and ground samples were ashed at 500 °C and dissolved in 1:30 (v/v) HCl. The concentration of P was determined colorimetrically according to Gericke and Kurnies (1952), while concentration of Zn was determined by atomic absorption spectrophotometry (AAS).

3.4. Results

Leaf symptoms and shoot dry matter production

The first visible characteristic reaction of plants to Zn deficiency was the decrease in shoot elongation and leaf area. This characteristic symptom of Zn deficiency occurred firstly and more noticeably in durum wheat cv. Kunduru-1149. Shoot dry weights and the calculated Zn efficiency values are given in *Tables 3.2 and 3.3*. Rye cv. Aslim showed the least reduction in shoot dry weight under Zn deficiency. Among the bread wheats, there was no difference in dry matter production after 23 days growth and as a result of this, they had similar Zn efficiency values. However, in the second harvest, they became different in such a way that cv. Bezostaja showed less reduction in shoot dry matter under Zn deficiency than cv. BDME-10.

Table 3.2. Effect of Zn supply (+Zn= 5 mg kg^{-1} soil) on shoot dry weight and Zn efficiency of different cereal genotypes grown for 23 days in a soil with low Zn availability. The data represent mean±SD from four independent replications

Genotype	Shoot dry weight (g $plant^{-1}$)		Zn efficiency (%) *
	-Zn	+Zn	
Secale cereale			
Aslim	0.36±0.01	0.43±0.01	84
Triticum aestivum			
Bezostaja	0.25±0.01	0.37±0.02	68
BDME-10	0.26±0.01	0.38±0.03	68
Triticum durum			
Kunduru-1149	0.25±0.02	0.44±0.02	57

* Efficiency= (Dry weight at –Zn/Dry weight at +Zn) x 100

The reduction in shoot elongation was followed by the development of whitish-brown necrotic areas (patches) on middle parts of leaves and this was also a typical characteristic of plants grown under Zn deficiency (*Picture 3.1*). The genotypes used in this study considerably differed in time of appearance and severity of such Zn deficiency symptoms. Among the genotypes, the durum wheat cv. Kunduru-1149 was the first one that showed the necrotic patches after 21 days growth as well as the reduction in shoot dry matter production (*Tables 3.2 and 3.3*). Among bread wheats, these symptoms were first and severer seen in cv. BDME-10 whereas the other bread wheat cv. Bezostaja was less affected (*Table 3.3*). Zinc deficiency symptoms were either absent or only slightly developed in the rye cv. Aslim.

Table 3.3. Effect of Zn supply (+Zn= 5 mg kg^{-1} soil) on leaf symptoms of Zn deficiency, shoot dry weight and Zn efficiency of different cereal genotypes grown for 30 days in a soil with low Zn availability. The data represent mean±SD from four independent replications

Genotype	Leaf Symptoms*	Shoot dry wt. (g $plant^{-1}$) -Zn	Shoot dry wt. (g $plant^{-1}$) +Zn	Zn Efficiency** (%)
S. cereale				
Aslim	5	0.72±0.02	0.93±0.04	77
T. aestivum				
Bezostaja	3	0.49±0.01	0.84±0.05	58
BDME-10	2	0.45±0.02	0.82±0.02	49
T. durum				
Kunduru-1149	1	0.43±0.01	0.92±0.01	47

* Leaf symptoms of Zn deficiency (necrotic patches on leaf blade): 1=very severe, 2=severe, 3=mild, 4=moderate, 5=very slight or absent

** Efficiency= (Dry weight at –Zn/Dry weight at +Zn) x 100

Concentration of Zn, P, and Fe

The concentration of Zn in shoot dry matter was increased by Zn application in all genotypes (*Figure 3.1*). Without Zn supply, the genotypes did not differ very much in their Zn concentration in shoot. Only Zn deficient rye tended to have slightly higher Zn concentration in both harvests than the other genotypes. Among the wheat genotypes, the durum wheat cv. Kunduru-1149 had the lowest Zn concentration (*Figure 3.1*).

As shown in *Figure 3.2*, the concentration of P in shoot dry matter was enhanced as the severity of Zn deficiency stress increased. All wheat genotypes showed this reaction but in the durum wheat cv. Kunduru-1149 was more distinct, particularly in the first harvest. On the contrary, P concentration of the rye cv. Aslim was not affected by different Zn supplies and was constant.

The shoot concentration of Fe decreased with Zn application in all genotypes, in particular in the second harvest (*Table 3.4*). But there was no relationship between Fe concentrations and Zn efficiency values of genotypes given in *Tables 3.2 and 3.3*.

Content of Zn and P in shoot

As a result of the increases in shoot dry matter production and Zn concentration, the plants supplied with Zn have a greater amount of Zn in shoots (*Figure 3.3*). Under Zn deficiency, genotypes tended to differ in the amount of Zn accumulation in shoots. Compared to the wheat genotypes, the rye cv. Aslim accumulated much more Zn in the shoot when not treated with Zn. Among the wheat genotypes, the bread wheat cv. Bezostaja had more Zn in the shoot than the others (*Figure 3.3*). The amount of Zn in shoot correlated better with Zn efficiency of genotypes than the concentration (*Figure 3.4*).

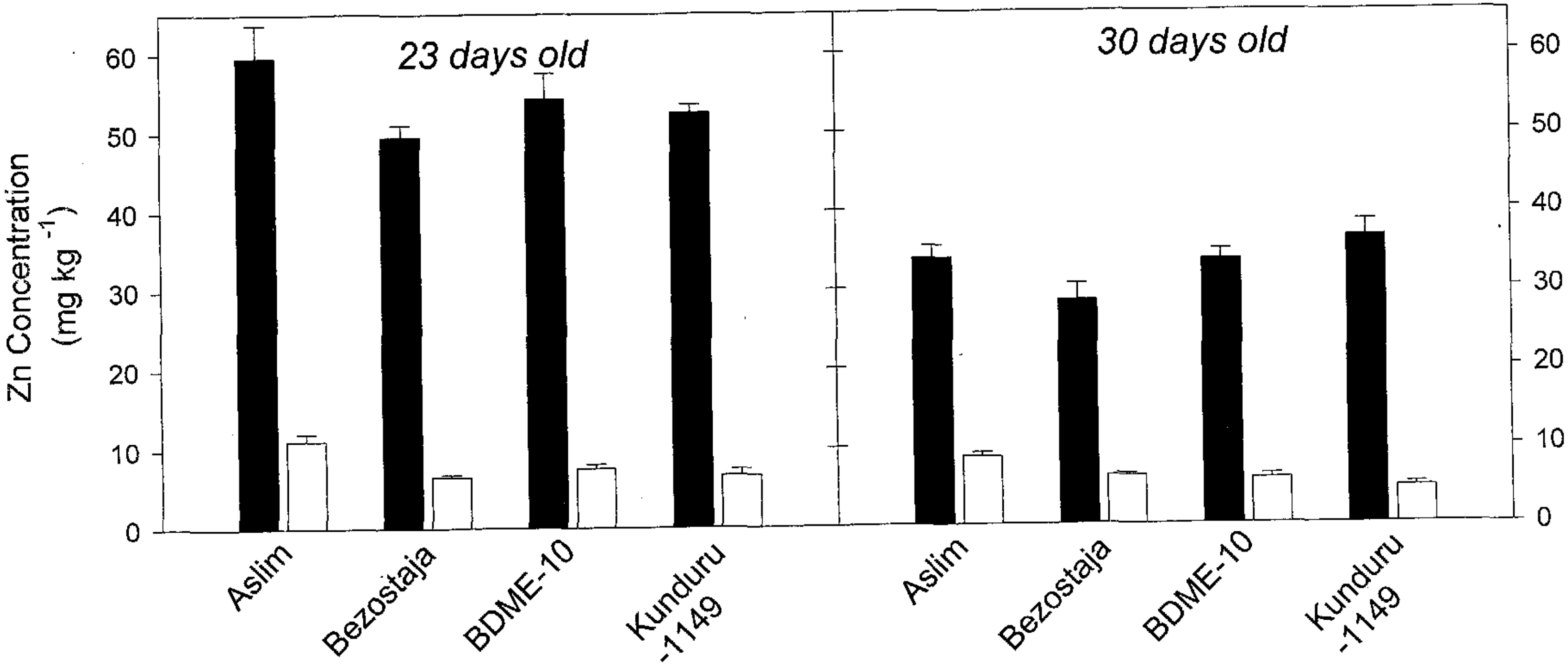


Figure 3.1. Effect of Zn supply (+Zn = 5 mg Zn kg^{-1} *soil*) on the concentration of Zn in shoots of different cereals (Rye cv. Aslim; Bread wheat cvs. Bezostaja and BDME-10; Durum wheat cv. Kunduru-1149) grown for 23 and 30 days in a soil with low Zn availability. Data represent means ± SD from four independent replications

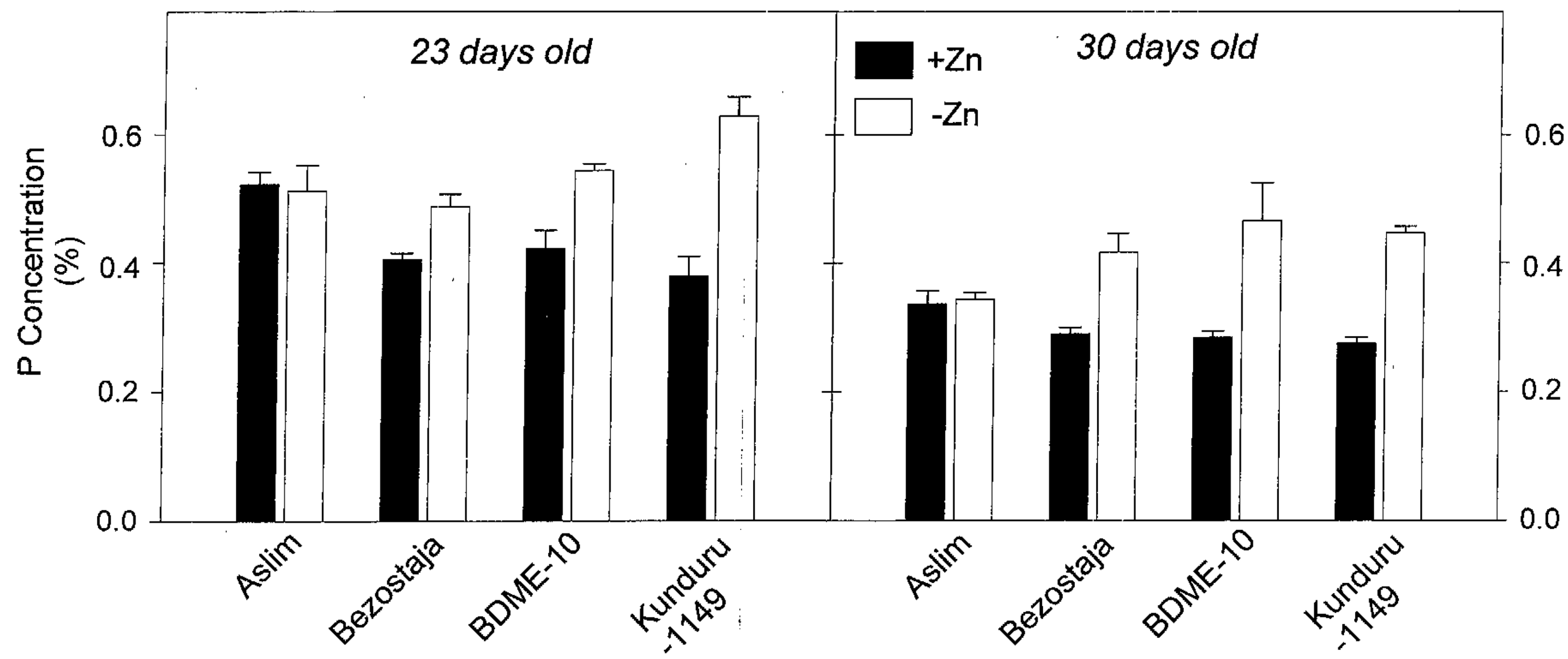


Figure 3.2. Effect of Zn supply (+Zn = 5 mg Zn kg^{-1} soil) on the concentration of P in shoots of different cereals (Rye cv. Aslim; Bread wheat cvs. Bezostaja and BDME-10; Durum wheat cv. Kunduru-1149) grown for 23 and 30 days in a soil with low Zn availability. Data represent means ± SD from four independent replications

Table 3.4. Effect of varied Zn supply (+Zn= 5 mg kg^{-1} soil) on concentration of Fe in shoot of different cereal genotypes (rye, bread wheat, and durum wheat) grown for 30 days in a soil with low Zn availability. The data represent means±SD of four independent replicates

	Fe Concentration ($mg\ kg^{-1}$ dry wt.)			
	23 days old		30 days old	
Genotype	-Zn	+Zn	-Zn	+Zn
Secale cereale				
Aslim	87±15	76±3	130±25	55±3
Triticum aestivum				
Bezostaja	87±11	80±23	110±2	45±1
BDME-10	132±18	65±2	159±30	46±6
Triticum durum				
Kunduru-1149	148±34	61±9	121±5	54±15

Despite 44-73% increases in shoot dry matter on average of all genotypes with Zn application, respectively in the first and second harvests, the content of P in the shoot increased only 16-26% (*Figure 3.5*).

P/Zn ratio in shoot

The genotypes had different P/Zn ratios without Zn supply but not with Zn supply (*Figure 3.6*). As expected, the Zn sufficient plants showed less P/Zn ratios than the Zn-deficient plants. Under Zn-deficient conditions, P/Zn ratio of the rye cv. Aslim was the closest to the ratios of plants supplied with adequate Zn and the ratio of the durum wheat cv. Kunduru-1149 had the most distance. Among the bread wheat genotypes, Bezostaja with higher Zn efficiency value showed lower P/Zn ratio than BDME-10.

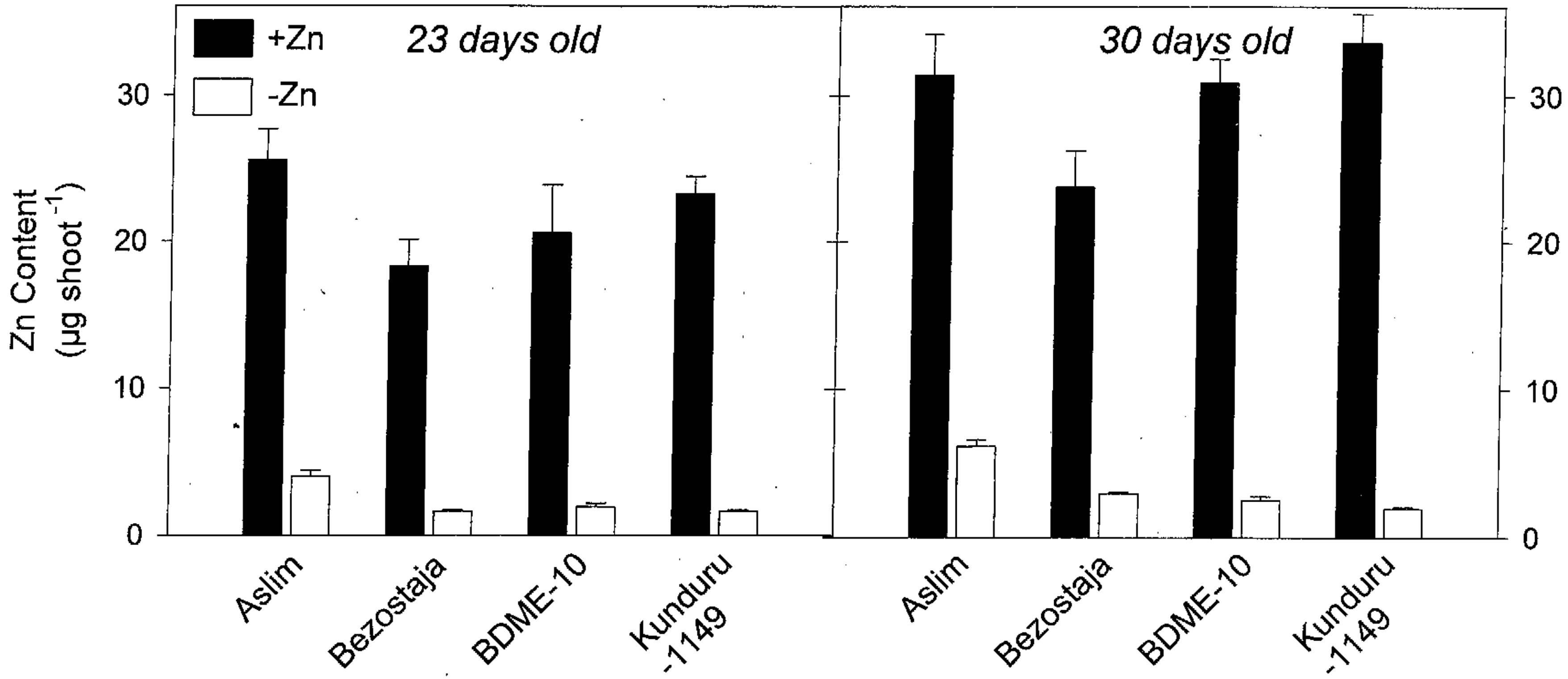


Figure 3.3. Effect of Zn supply (+Zn = 5 mg Zn kg^{-1} soil) on the amount (content) of Zn in shoots of different cereals (Rye cv. Aslim; Bread wheat cvs. Bezostaja and BDME-10; Durum wheat cv. Kunduru-1149) grown for 23 and 30 days in a soil with low Zn availability. Data represent means ± SD from four independent replications

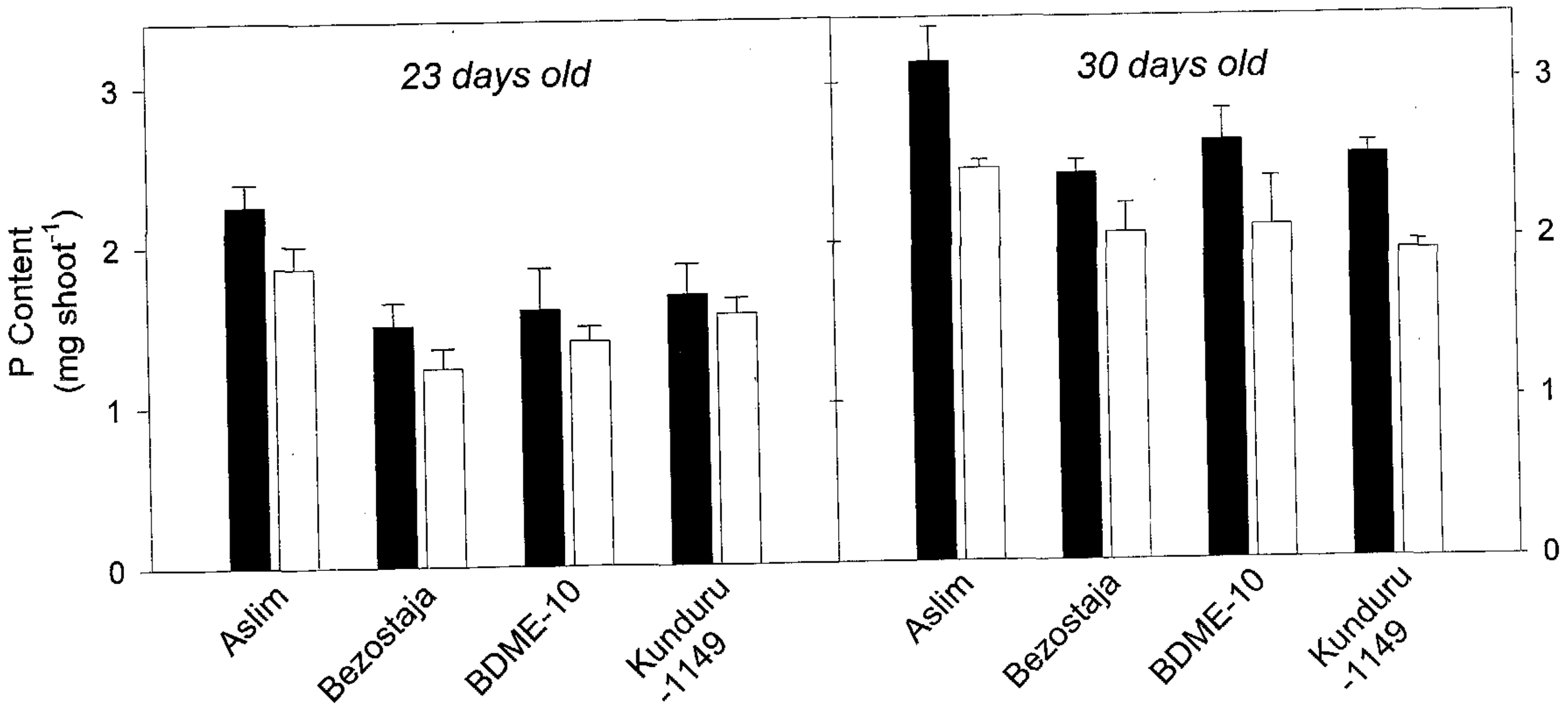


Figure 3.4. Effect of Zn supply (+Zn = 5 mg Zn kg^{-1} soil) on the amount (content) of P in shoots of different cereals (Rye cv. Aslim; Bread wheat cvs. Bezostaja and BDME-10; Durum wheat cv. Kunduru-1149) grown for 23 and 30 days in a soil with low Zn availability. Data represent means ± SD from four independent replication

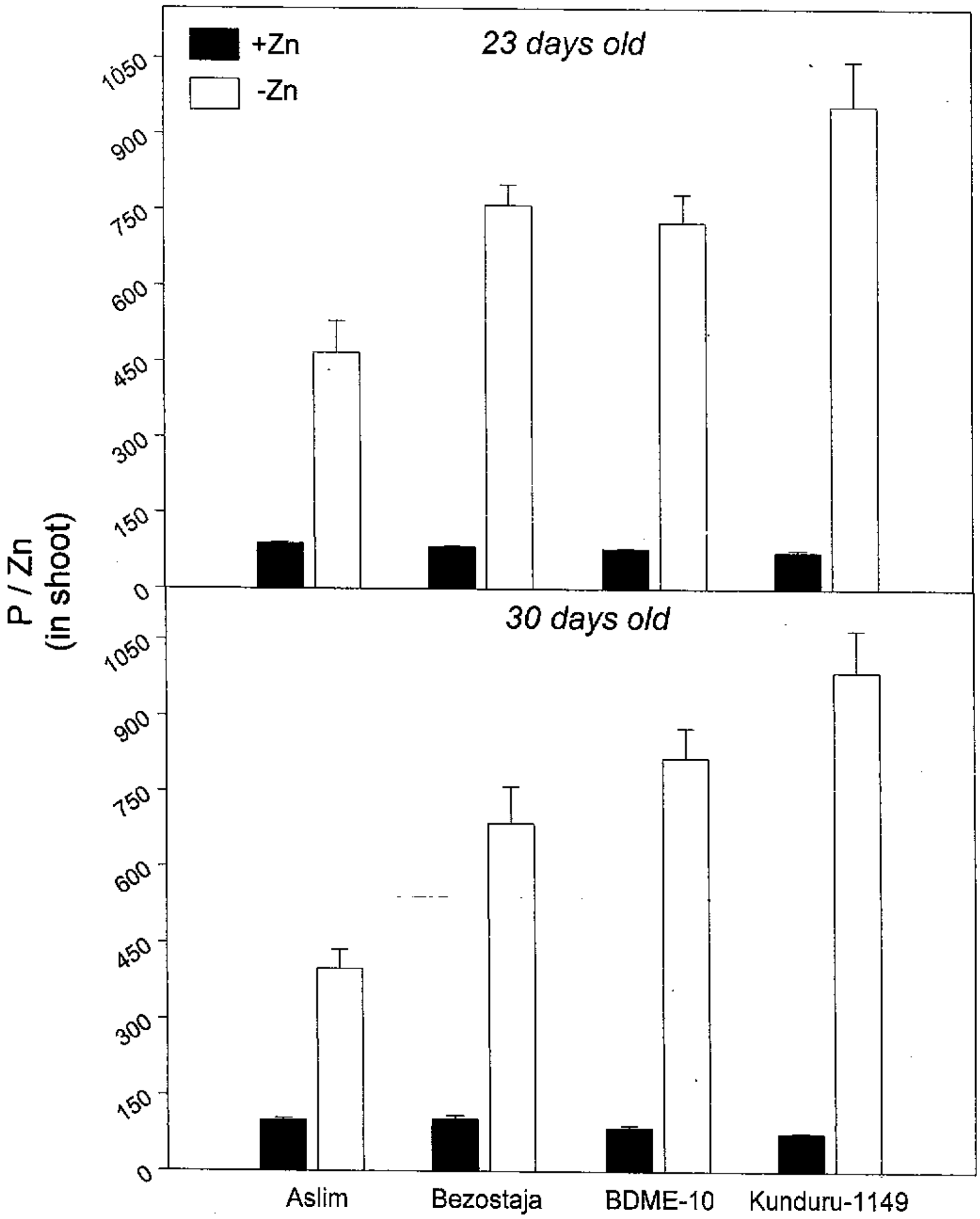


Figure 3.5. Effect of Zn supply (+Zn = 5 mg Zn kg^{-1} soil) on the P/Zn ratio in shoots of different cereals (Rye cv. Aslim; Bread wheat cvs. Bezostaja and BDME-10; Durum wheat cv. Kunduru-1149) grown for 23 and 30 days in a soil with low Zn availability. Data represent means ± SD from four independent replications

3.5. Discussion

Sensitivity to Zn deficiency varied widely among genotypes used in this study, particularly the rye cv. Aslim was the most efficient one, while the durum wheat cv. Kunduru-1149 was the most sensitive to Zn deficiency (*Tables 3.2 and 3.3*). Bread wheats also differed in susceptibility to Zn deficiency, particularly after the first harvest. The bread wheat cv. Bezostaja was less affected by Zn deficiency comparing to the other bread wheat cv. BDME-10 (*Tables 3.2 and 3.3*). These results are in well-accordance with earlier studies (Cakmak et al., 1996a, 1998). Cakmak et al. (1998) showed that the resistance of cereals to Zn deficiency grown on a Zn-deficient calcareous soil increased in the order of rye > triticale > barley > bread wheat ≥ oat > durum wheat.

There was no clear relationship between the differential susceptibility of genotypes to Zn deficiency and the Zn concentration in shoot (*Figure 3.1*). As mentioned previously by other researchers (Cakmak et al., 1996a, b, 1998; Graham et al., 1992), tissue concentration of Zn in shoot is not a good parameter for distinguishing resistance to Zn deficiency among genotypes. Only in the case of rye cv. Aslim, there was a partial correlation between high Zn efficiency of this cultivar and high Zn concentration in shoot (*Figure 3.1*). However, the sensitivity of genotypes was better related with the content of Zn per shoot (*Figure 3.2*). The highest accumulation was found in the rye cv. Aslim, the most resistance to Zn deficiency and the lowest in the most sensitive one, the durum wheat cv. Kunduru-1149. In view of these results, it seems very likely that the expression of high Zn efficiency in cereals, as mentioned before, is associated with enhanced capacity for Zn acqusition. The same researchers (Cakmak et al., 1996a, b, 1998; Graham et al., 1992) attributed the similar Zn concentrations in shoot tissues of genotypes differing in Zn efficiency to the dilution of Zn by growth. Under deficient supply of Zn, a Zn-efficient genotype enhances uptake of Zn, and which in turn increases shoot dry matter production resulting in a corresponding dilution of Zn in tissues to similar concentration as in the inefficient one.

The high Zn efficiency of rye was attributed to a high uptake capacity for Zn in the short-term uptake experiments conducted in nutrient solution (*Chapter 7*; Cakmak et al., 1998; Erenoglu et al., 1999). Although the bread and durum wheat genotypes differed in Zn uptake rates from nutrient solution in a short-term experiment conducted by Cakmak et al. (1998), in an another short-term experiment which was also carried out in nutrient solution as short-term experiment, the bread wheat genotypes did not show any difference in Zn uptake (*Chapter 7*; Erenoglu et al., 1999). In contrast, it was already shown that the bread and durum wheat genotypes differ in their Zn uptake rates from nutrient solution and these differential uptake rates are in well-agreement with their Zn efficiency values (Hart et al., 1998; Rengel & Graham, 1996; Rengel et al., 1998). More recently, using one Zn efficient and one Zn inefficient bread wheat cultivars used by Erenoglu et al. (1999) and in *Chapter 7*, Hacisalihoglu et al. (2001) presented that there are two different Zn transport systems mediating high- and low-affinity Zn influx for both cultivars. However, they also could not find any difference between both genotypes neither in low-affinity nor in high-affinity activity ranges (Hacisalihoglu et al., 2001). However, these contradictory results on the relationship between Zn efficiency and Zn uptake of bread wheat cultivars and partially of durum wheats in nutrient solution should not underestimate that uptake of Zn is completely not important in differential Zn efficiency of cereals, because the equilibrium between plants and nutrients are more complicated in the soil than in the nutrient solution. As seen in coming chapter, the amount of Zn translocated from roots to shoot could also vary in the soil and the nutrient solution culture. Special interest should be given on this point in future studies. Since, while in coming chapter, very high accumulation of Zn in roots in soil conditions was measured, Zn concentrations in shoot and roots of plant materials in *Chapter 6* and root-to-shoot translocation rates of Zn in wheat cultivars in *Chapter 7*, show well partitioning of Zn between roots and shoot.

The increased P concentration in the shoot tissues under Zn deficiency is a well-known phenomena in both monocotyledon and dicotyledon plant species (Welch et al., 1982; Cakmak & Marschner, 1986; Webb & Loneragan, 1988; Welch & Norvell, 1993) and can reach levels which are considered as toxic to plants if high concentrations of P are supplied (Loneragan et al., 1982; Welch et al., 1982; Cakmak & Marschner, 1986; Norvell & Welch, 1993). The results of the experiment presented in this chapter show that the cereal genotypes grown without Zn supply increased the P concentration in shoot tissue with exception of rye cv. Aslim having the highest Zn efficiency and no necrotic patches on leaf blades did not change with the external supply of Zn (*Figure 3.2*). An impaired feedback mechanism in the shoots of Zn-deficient cotton which controls the P uptake by roots and especially the P transport from roots to shoots was introduced (Marschner & Cakmak, 1986). Recently, it has been also shown that, in Zn deficient barley roots, the expression of high-affinity phosphate transporter genes is up-regulated (Huang et al., 2000). All genotypes under Zn deficiency had higher P/Zn ratio values than the value 150 which was found in grape leaves as a critical level of Zn deficiency (Marschner & Schropp, 1977), for example, in 30 days old plants it ranged between 398 and 980. The P/Zn values obtained in this chapter show well-accordance with the susceptibility of genotypes to Zn deficiency. Consequently, it may be concluded that P/Zn ratio in shoot tissue is a useful parameter to express the differential Zn efficiency in cereals, as mentioned by Bergmann (1993).

Increasing Zn supply to the roots has been shown to increase (Jolley & Brown, 1991), have little effect on (Norvell & Welch, 1993) or decrease (Safaya, 1976; Jolly & Brown, 1991) Fe concentration in shoots. However, increasing Fe, in general, has only a depressive effect on Zn concentration in plant tissues (Zhang et al., 1991). The results presented here show that plants grown without Zn supply increased the shoot tissue Fe concentration compared to plants supplied with sufficient Zn (*Table 3.4*). However, there is no relationship between the tissue concentrations of Fe and Zn efficiency of cultivars used in this study.

According to the data of some earlier researches, PS's that are efficient in complexing and mobilising Zn in both soils and plants might increase root uptake and root-to-shoot translocation of Zn in Zn-efficient cereals (Cakmak et al., 1994, 1996c; Treeby et al., 1989; Welch, 1995; Zhang et al., 1991). However, as mentioned in *Chapter 5* and *6* of this PhD thesis and published elsewhere (Erenoglu et al., 1996; Cakmak et al., 1998), release rate of PS's from roots is not well related with Zn efficiency of cereals in nutrient solution, with the exception of less PS's release rates of durum wheats which are, in general, considered as Zn-inefficient.

Since the amounts of Zn per unit dry weight of shoot tissue do not differ among the cultivars, it could also be argued that differences in the internal utilization of Zn contribute to the expression of Zn efficiency. In good agreement with this, Cakmak et al. (1998) found greater activity of Zn-containing SOD in leaves of Zn efficient than inefficient genotypes, indicating a better utilization of Zn at the cellular level in efficient genotypes. Similarly, under Zn deficiency and at similar leaf concentrations of Zn, a higher activity of the Zn-containing carbonic anhydrase enzyme was measured in leaves of Zn efficient compared with inefficient wheat genotypes (Rengel, 1995).

In conclusion, Zn deficiency can cause significant reductions in shoot growth of cereals and cereal species and cultivars within a species differ in their susceptibility to Zn deficiency. The total amount of Zn and P/Zn ratio in shoot are better indicator for differential Zn efficiency of cereals than the tissue concentration of Zn.

4. Role of arbuscular mycorrhiza (AM) in growth and Zn and P uptake of wheat genotypes differing in Zn efficiency on calcareous soil with low availability of Zn

4.1. Abstract

Using two bread wheat (*Triticum aestivum* cvs. Bezostaja and BDME-10) and one durum wheat (*Triticum durum* cv. Kunduru-1149) cultivars, a greenhouse experiment was conducted to study the relationship between arbuscular mycorrhiza (AM), Zn efficiency, and Zn and P uptake over a 35 day growth period in a calcareous soil with extremely low Zn availability treated with different P, Zn, and AM. In general, the presence of mycorrhizal colonization had either no or negative effects on the plant growth. The presence of mycorrhizal colonization also increased the severity of leaf symptoms of Zn deficiency at high P and no Zn treatment, regardless of genotypical differences. The disturbed carbohydrate metabolism and/or partitioning in wheat cultivars is discussed as a possible reason for the hindered plant growth and enhanced Zn deficiency stress. Phosphorus deficiency increased the rate of AM colonization in the roots of all cultivars, irrespective of genotypical differences in Zn efficiency. The cultivars did not show any genotypival differences in the percentage of infected root part with AM at both P supply. While, as mentioned in literature, the wheat cultivars could enhance the uptake of P at the presence of mycorrhizal colonization, the Zn uptake was either not affected (+Zn) or even decreased (-Zn) with mycorrhizal colonization. It was observed that the wheat cultivars having different Zn efficiency could mobilize different amounts of Zn from soil

under Zn deficiency and this different mobilization tended to correspond with the susceptibilities of cultivars to Zn deficiency.

4.2. Introduction

Zinc (Zn) is one of the micronutrients, the deficiency of which is very widespread throughout the world (Sillanpää, 1982). A number of soil factors such as alkaline pH, high $CaCO_3$, poor drainage conditions, high Fe and Al hydroxides content, and heavy doses of P fertilizers are known to increase the Zn deficiency in crops (Sharma et al., 1994).

Arbuscular mycorrhiza are a group of fungi having endotrophic associations with the roots of most higher plants except those belonging to the Order Centrospermae and in the families Brassicaceae, Fumariaceae, Cyperaceae, and Commelinaceae (Gerdemann, 1975). Factors such as soil fumigation, low temperatures, wet soils, and P fertilization could negatively affect the fungal infection and/or efficiency in increasing nutrient uptake (Lambert et al., 1979).

The fungal partner acts similarly to root hairs, but may reach the soil volumes almost 7 cm far from root surfaces, increasing the volume of soil from which nutrients can be extracted (Lambert et al., 1979). The physiological importance of arbuscular mycorrhizal association for the higher plants is increased nutrient uptake, water absorption, drought tolerance, and root disease resistance (Gianinazzi-Pearson & Gianinazzi, 1983; Hayman, 1983). This function is especially important for nutrients which have limited diffusion zones around roots, particularly for P, Zn, and Cu. An increased uptake of phosphates (Bowen et al., 1975; Sanders et al., 1975; Abbott & Robson, 1977), Zn and Cu (Lambert et al., 1979; Swaminathan & Verma, 1979; Gnekow & Marschner, 1989; Kothari et al., 1990; Li et al., 1991). At high P levels, the arbuscular mycorrhizal association also provides sufficient uptake of Zn and Cu by plants (Lambert et al., 1979; Swaminathan

& Verma, 1979; Gnekow & Marschner, 1989) and therefore prevents P-induced deficiency of these micronutrients. However, in a study conducted using chickpea Weber and his colleagues (1993) showed a negative effect of AM on Zn nutritional status of plants. The dilution of Zn in shoot tissue due to increased growth of AM-inoculated plants was discussed as a possible reason for this decrease in Zn concentration (Weber et al., 1993). According to Marschner (1993), soils with low Zn amounts a decrease in mycorrhizal infection in roots is often associated with reduce in Zn contents in the shoots which exceed values that can be explained by dilution.

The Zn-dependent enzymes can play important roles in carbohydrate metabolism at various levels (Cakmak, 1988). An increasing Zn stress causes a sharp declines in carbonic anhydrase (CA) activity (Ohki, 1976) and this decline is the clearest change in activity of enzymes of carbohydrate metabolism (Shrotri et al., 1983). Normally, CA is present in the cytoplasm and chloroplasts and may speed up the transfer of CO_2/HCO_3 for photosynthetic CO_2 fixation (Römheld & Marschner, 1991). Carbonic anhydrase is present in leaves of higher plants in abundant quantities (1-2% of total soluble leaf protein; Okabe et al., 1984) and can thus represent a significant storage pool of Zn in leaf cells. Generally, CA is present in excess of what may be required for photosynthesis (Randall & Bouma, 1973), resulting usually in a relatively poor relationship between CA activity and photosynthetic rate. However, since CA activity is much lower in wheat comparing to a number of other species (Makino et al., 1992), the level of CA activity may be more important for maintaining the photosynthetic rate in wheat than in other species (Rengel, 1995). Some interesting results were obtained in studies conducted in recent years to clarify the relation between photosynthetic parameters and Zn nutritional status of plants. For example, in rice with low Zn supply, marked reductions in growth and photosynthetic processes such as Hill reaction activity, photophosphorylation rate, thylakoid phosphorylation, and CO_2-fixation were found compared to those with sufficient supply (Ajay & Rathore, 1995). Moreover, the effect of Zn nutrition on CO_2 gas exchange (Fischer et al., 1997) and on CA activity (Rengel, 1995) in leaves of wheat genotypes differing in Zn efficiency were studied in recent years.

The topic of the interaction between Zn and P has been discussed frequently as reviewed by Loneragan and Webb (1993). But it is still a matter of discussion due to so many confusing results. One of the discussion points in this P-Zn interaction is the P/Zn ratio and the role in appearance of Zn deficiency symptoms. For example, it was found in grape leaves with critical values of around 150 for plants grown in soil (Marschner & Schropp, 1977). In addition, the good correlation between leaf symptoms and as well leaf P concentrations as the ratio of concentrations P/Zn led to the examination of the relation between Zn deficiency and P concentrations in plants (Loneragan & Webb, 1993). Plants normally control their capacity to take up P to maintain the P concentration in shoot tissue within physiological limits. Therefore, they decrease the speed of P uptake when grown under high-P conditions but increase this capacity when grown under low-P conditions (Clarkson & Scattergoor, 1982; Jungk et al., 1990). However, when exposed to low levels of Zn, high amounts of P are accumulated in the tissues of both dicots and monocots (Welch et al., 1982; Cakmak & Marschner, 1986; Webb & Loneragan, 1988; Welch & Norvell, 1993). This suggests that Zn-deficient plants lose control over the absorption mechanism (Safaya & Gupta, 1979; Marschner & Cakmak, 1986). Recently, it has been shown that Zn deficiency increases the expression of high-affinity P transporter in barley roots (Huang et al., 2000).

The aims of present chapter were (i) to evaluate the role of AM in Zn and P uptake and (ii) to investigate the relationship between mycorrhizal infection and Zn efficiency of bread and durum wheat genotypes grown on an extremely Zn-deficient soil.

4.3. Materials and methods

Two bread wheat (*Triticum aestivum* L. cvs. Bezostaja and BDME-10) and one durum wheat (*Triticum durum* L. cv. Kunduru-1149) were used in the experiment conducted in greenhouse conditions. This experiment was set up to determine the influence of P supply and AM on Zn uptake, severity of Zn deficiency stress, and P/Zn ratios in the plants grown on a severe Zn-deficient soil.

Preparation of VA mycorrhizal inoculum

Since the solubility of most of soil-nutrients, including Zn, is increased by chemical and heating sterilization, the soil used in this study were sterilized by gamma radiation (25 kGy, Company Rütsch, Stuttgart, Germany). Some physical and chemical properties of the soil were given in *Chapter* 3. DTPA-extractable Zn with and without γ-sterilization are given in *Table 4.1*. Mycorrhizal inoculum was prepared by wet-sieving soil from the root systems of maize (*Zea mays* L. cv.) colonized with the mycorrhizal fungus *Glomus intraradices* to obtain fungal spores falling in the 20 µm range and inoculated root particles. Thereafter, the fungal spores obtained and the infected root particles were added to the soil sterilized by gamma radiation. Then, the soil was thoroughly mixed with 200 mg N kg^{-1} soil (as $Ca(NO_3)_2$), 20 mg P kg^{-1} soil (as KH_2PO_4) and 2 mg Fe^{III}-EDTA kg^{-1} soil. Maize seeds were sown subsequently into this mixture and the plants were grown for 8 weeks. At the end of the 8 weeks period, the plant shoots were removed and the roots were sieved to 2 mm and colonized root particles were cut into smaller (1-2 cm) pieces. Thereafter, the sieved soil and the cut root particles were mixed homogeneously and used in the main part of experiment. The AM infection rate of the roots in inoculum was 70-75%.

Table 4.1. Effect of γ-sterilization on the DTPA-extractable Zn of the calcareous soil from Central Anatolia-Turkey

DTPA-extractable Zn ($mg\ kg^{-1}$ soil)	
with	without
0.100	0.095

Experiment 1: Role of AM in Zn efficiency of bread and durum wheats

Plants were potted into 650 g of soil. The experimental design is summarized in *Table 4.2*. The amounts N and Fe(III)-EDTA were as given in *Preparation of VA mycorrhizal inoculum.* All nutrients and AM were mixed throughly with the sterilized soil. For the treatments with AM, 585 g of the soil was mixed with 65 g of the inoculum. Non-mycorrhizal treatments received a filtrate of 65 g portion of the inoculum. This filtrate did not contain mycorrhizal propogules but did contain the same bacterial population as in the mycorrhizal treatments.

Table 4.2. Zinc and P treatments used in *Experiment 1*

Treatments	Zn ($mg\ kg^{-1}$ soil)	P
-Zn/low P	0	20
-Zn/high P	0	100
+Zn/low P	5	20
+Zn/high P	5	100

Each treatment had four replicates. Eight seeds were sown in each pot; the seedlings were thinned to four plants per pot after germination. All pots were watered twice in a day to field capacity using deionized water. Plants were harvested 35 days after planting. The roots and shoots were separated and root samples were carefully washed with deionized water to remove soil particles from root surface and to reduce the risk of contamination to a minimum.

To determination of arbuscular mycorrhizal colonization, a part of the roots were stained with 0.1% acid fuchsin in lactic acid for 30 minutes at room temperature after clearing in 10% KOH at 80 °C for 2 hours and bleaching in alkaline H_2O_2 at room temperature overnight. After staining, the roots were destained in lactic acid.

Dry weights of the shoots and roots were determined after drying at 70 °C. Determination of the concentrations of P and Zn was as described in the Chapter 3.

4.4. Results

Mycorrhizal infection in roots

The percentage (%) of mycorrhizal infection in the roots of wheat cultivars grown for 35 days on a Zn-deficient soil with and without mycorrhizal inoculation is given in *Table 4.3*. As expected, the roots of plants inoculated with no AM, did not have any mycorrhizal infection in all treatments (*data not shown*). In all cultivars, plants supplied with low P had a higher mycorrhizal colonization of the root compared to plants supplied with high P (*Table 4.3*). At both P levels, Zn sufficient plants had higher mycorrhizal colonization (*Table 4.3*).

Visual observations of shoots and shoot and root dry matter production

The plants supplied with no Zn and low P did not have whitish-brown necrotic patches on the middle parts of leaves. The latter symptom is the second characteristic reaction to occur in plants with Zn deficiency (after the reduction in shoot elongation and leaf size). However, plants grown at -Zn/high P treatment differed in the timing of appearance and severity of the Zn deficiency symptoms depending on mycorrhizal treatment (*Table 4.4*). All cultivars without mycorrhizal inoculation were less affected by Zn deficiency than the plants with mycorrhizal inoculation (*Table 4.4*). Also, the cultivars differed in the severity of the deficiency symptoms at both mycorrhizal tretments (*Table 4.4*). For example, while in both cases durum wheat cv. Kunduru-1149 was the most sensitive one, bread wheat cv. Bezostaja showed the least Zn deficiency symptoms on the leaf blade (*Table 4.4*).

Table 4.3. Effect of P and Zn supply on rate of mycorrhizal colonization in roots of bread and durum wheat cultivars grown for 35 days on a Zn-deficient soil with mycorrhizal infection. Data represent means±SE of four independent replicates

	Mycorrhizal colonization (%)			
	+Zn		-Zn	
Genotypes	High P	Low P	High P	Low P
Triticum aestivum				
Bezostaja	10.6 ± 2.0	75.3 ± 1.8	6.6 ± 1.8	39.0 ± 5.5
BDME-10	12.6 ± 2.6	83.6 ± 1.5	4.0 ± 0.8	54.0 ± 14.0
Triticum durum				
Kunduru-1149	4.3 ± 1.8	69.9 ± 4.3	5.0 ± 2.8	43.3 ± 3.2
Mean	9.2	76.3	5.2	45.4

At all treatments, mycorrhiza had no positive effect on either shoot or root growth and even though low infection rates in plants at -Zn/high P treatment (*Table 4.3*), shoot and root growth of these plants were negatively affected (*Figure 4.1*) during 35 days of growth in an extremely Zn-deficient soil. This negative effect of mycorrhizal infection was relatively similar in all cultivars. Also, the plants at +Zn/low P treatment had negative response (*Figure 4.1*) to the highest mycorrhizal colonization among all treatments (*Table 4.3*).

Table 4.4. Leaf symptoms of Zn deficiency in bread and durum wheat cultivars grown for 35 days on a Zn-deficient soil with low or high P and with and without mycorrhizal infection

	Leaf symptoms*			
	+VAM		-VAM	
Gentotypes	high P	low P	high P	low P
Triticum aestivum				
Bezostaja	3	5	4	5
BDME-10	2	5	3	5
Triticum durum				
Kunduru-1149	1	5	1-2	5

* Leaf symptoms of Zn deficiency (necrotic patches on leaf blade): 1=very severe, 2=severe, 3=mild, 4=moderate, 5=very slight or absent

Responses of cultivars to sufficient Zn or P supply was positive in such a way that increasing supplies of both nutrients caused a rise in yield (*Figure 4.1*). The Zn efficiency values of cultivars determined by calculating the ratio of yield in -Zn treatment to yield in +Zn treatment and expressed in percentage, were much higher in the plants supplied with lower P than in the plants supplied with higher P (*Table 4.5*).

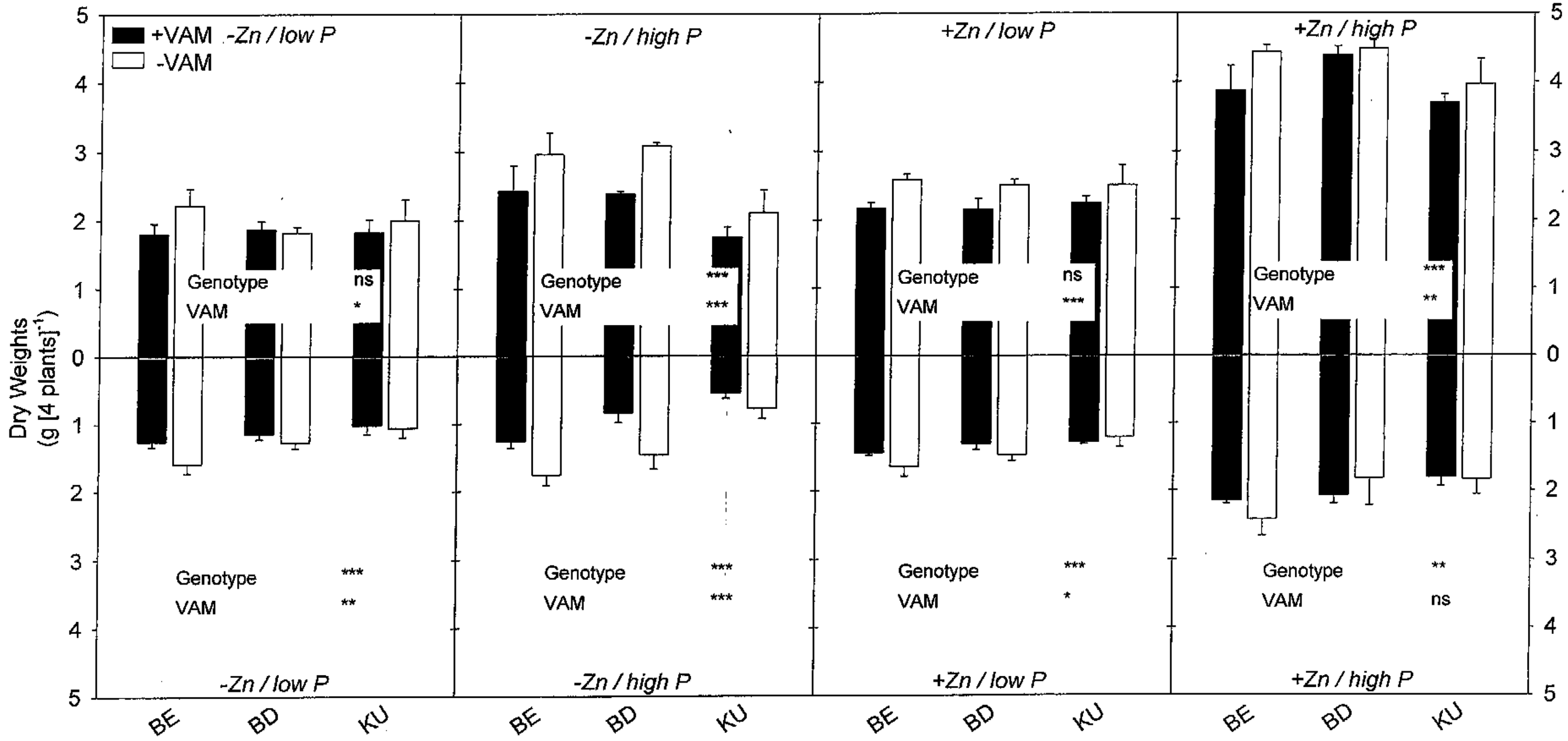


Figure 4.1. Effect of different Zn, P, and AM treatments on the growth of shoot (upper graphs) and roots (lower graphs) of bread (BE: Bezostaja, BD: BDME-10) and durum (KU: Kunduru-1149) wheat cultivars differing in Zn efficiency grown for 35 days in a Zn-deficient soil. The results represent means ± SE (vertical bars) of four independent replicates and were evaluated with two way ANOVA by using Sigma Stat software (***: $0.001<p$, **: $0.01<p$, *: $0.05<p$, and ns: not significant)

Table 4.5. Effect of Zn supply (+Zn= 5 mg kg^{-1} soil) on Zn efficiency of bread and durum wheat cultivars grown for 35 days on a calcareous soil containing low amount of plant available Zn, with low or high P and with and without mycorrhizal infection

	Zn Efficiency*, (%)			
	+VAM		-VAM	
Genotypes	high P	low P	high P	low P
Triticum aestivum				
Bezostaja	63	83	67	86
BDME-10	55	87	69	73
Triticum durum				
Kunduru-1149	48	82	53	80

* Efficiency= (Dry weight at -Zn/Dry weight at +Zn) x 100

Concentrations of Zn and P and P/Zn ratios in shoot and roots

The supply of Zn increased the shoot tissue concentration of Zn in all cultivars at both P levels and in mycorrhizal and non-mycorrhizal plants (*Figure* 4.2). The plants supplied with low P at both Zn levels tended to have higher tissue Zn concentrations in shoots than the plants at higher P treatments (*Figure* 4.2). For example, while the Zn concentrations in shoots of cultivars at +Zn/low P ranged from 51 to 78 μg g^{-1}, at +Zn/high P treatment the values between 30 and 48 μg g^{-1} were measured (*Figure* 4.2). Generally, the presence of mycorrhizal inoculum had no effect on the tissue Zn concentration in shoots of cultivars at all treatments (*Figure* 4.2). But in the cases of +Zn/low P and –Zn/low P where the mycorrhizal colonization was high (76.3 and 45.4%, respectively (*Table 1*)), the cultivars tended to contain little more Zn per shoot dry weight (*Figure* 4.2). However, in all cases the Zn concentration in roots either not affected or decreased with the presence of

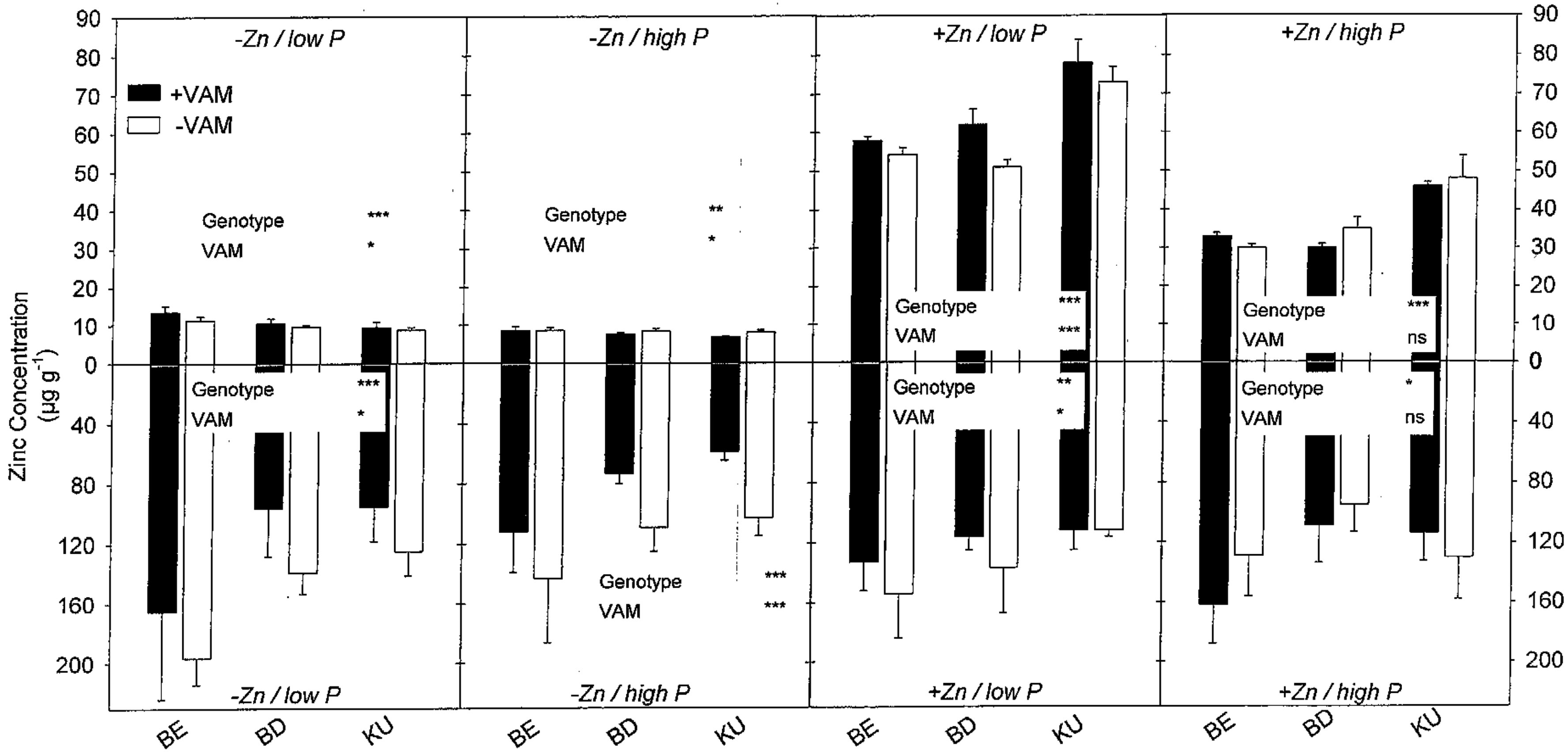


Figure 4.2. Effect of different Zn, P, and AM treatments on the concentration of Zn in shoot (upper graphs) and roots (lower graphs) of bread (BE: Bezostaja, BD: BDME-10) and durum (KU: Kunduru-1149) wheat cultivars differing in Zn efficiency grown for 35 days in a Zn-deficient soil. The results represent means ± SE (vertical bars) of four independent replicates and were evaluated with two way ANOVA by using Sigma Stat software (***: $0.001<p$, **: $0.01<p$, *: $0.05<p$, and ns: not significant)

mycorrhizal inoculum and this was more distinct under Zn deficient treatments (*Figure 4.2*). Generally, in Zn-deficient treatments, irrespective of P supply and mycorrhizal inoculation all cultivars tended to have Zn concentrations in roots in accordance with their Zn efficiency in field and greenhouse conditions (*Figure 4.2*).

If the cases in mycorrhiza were effective, are not taken into consideration, the Zn deficiency enhanced the P uptake by plants (*Figure 4.3*), especially the plants at -Zn/high P treatment which absorbed more P than the plants supplied with sufficient Zn and P (*Figure 4.3*). The presence of mycorrhizal inoculum had positive effect on P uptake in all cultivars, particularly in plants supplied with low P and having higher mycorrhizal infection rate (*Figure 4.3*). However, only in the case of +Zn/low P treatment having the highest infection rate, the P concentration in shoot was possitively affected by mycorrhizal colonization (*Figure 4.3*).

The P/Zn ratios in shoot and roots of wheat cultivars with different nutritional and VA mycorrhizal status are given in *Figure 4.4*. This ratio was generally higher in shoots than in roots (*Figure 4.4*). The plants enhanced the P/Zn ratio under deficient Zn supply compared to plants with sufficient Zn supply, particularly at high P level (*Figure 4.4*). The P/Zn ratio ranged between 132 and 167 for the Zn deficient plants with low P and between 635 and 883 for the Zn deficient plants with high P (*Figure 4.4*). However, for the Zn sufficient plants this ratio changed in a range between 17 and 32 at low P treatment and between 68 to 103 at high P treatment (*Figure 4.4*). The highest ratio in shoot was obtained in the case of durum wheat cv. Kunduru-1149 at -Zn/high P and at the same treatment bread wheat cvs. Bezostaja and BDME-10 had lower P/Zn ratios (*Figure 4.4*). In other treatments, the cultivars did not differ in this ratio (*Figure 4.4*). Besides, the presence of mycorrhizal inoculation had no effect on the P/Zn ratio in shoot, except for tendency noted at -Zn/high P (*Figure 4.4*). The effect of Zn

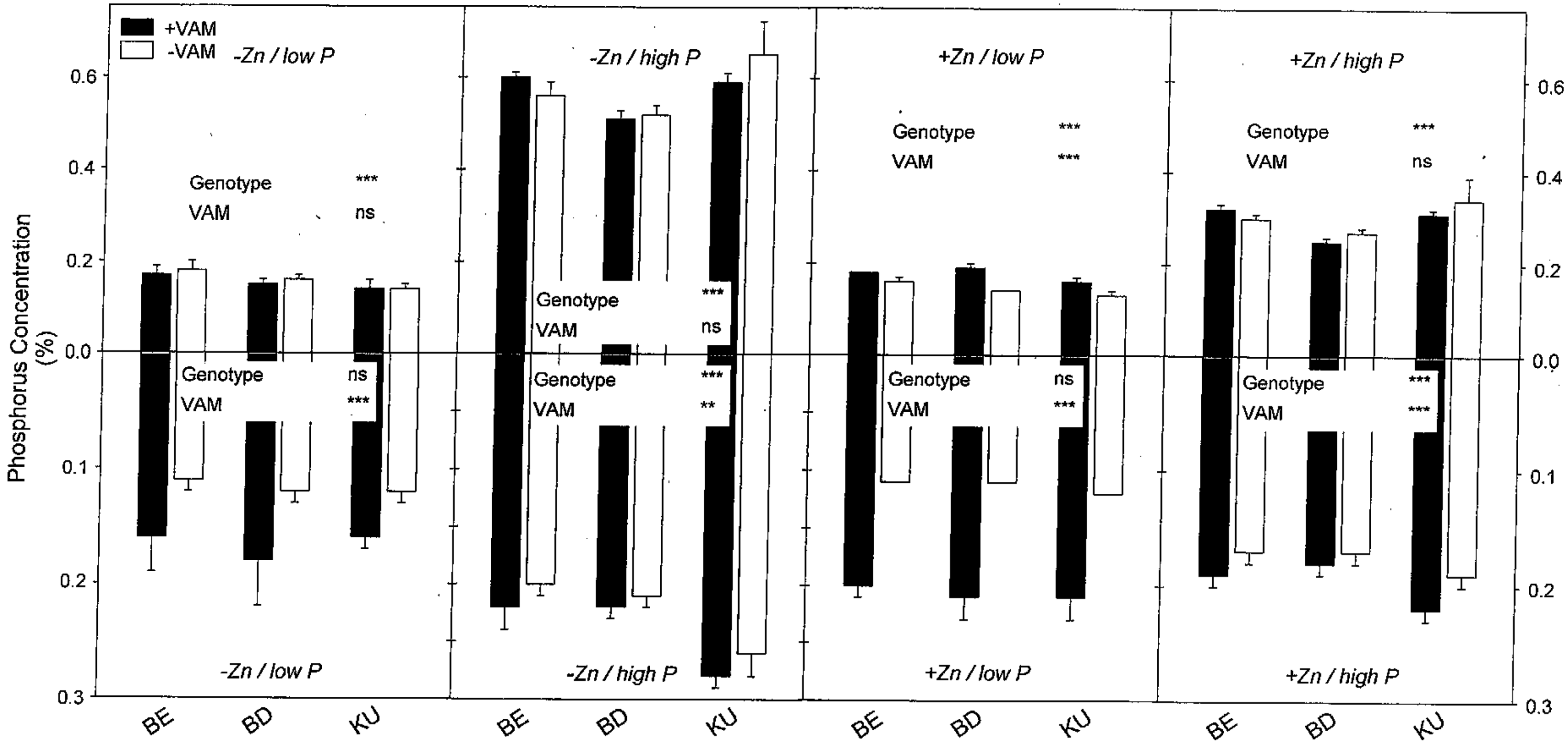


Figure 4.3. Effect of different Zn, P, and AM treatments on the concentration of P in shoot (upper graphs) and roots (lower graphs) of bread (BE: Bezostaja, BD: BDME-10) and durum (KU: Kunduru-1149) wheat cultivars differing in Zn efficiency grown for 35 days in a Zn-deficient soil. The results represent means ± SE (vertical bars) of four independent replicates and were evaluated with two way ANOVA by using Sigma Stat software (***: 0.001<p, **: 0.01<p, *: 0.05<p, and ns: not significant)

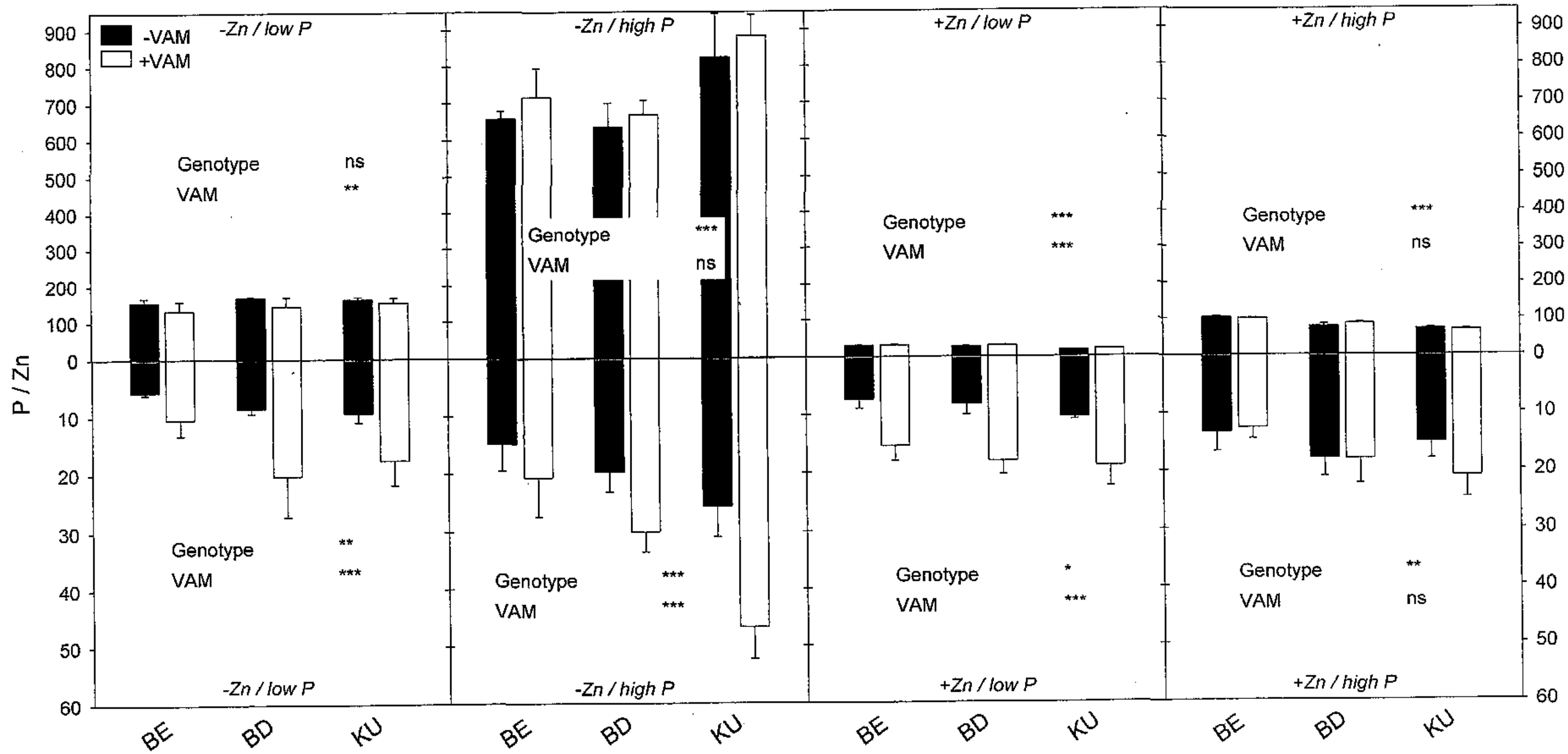


Figure 4.4. Effect of different Zn, P, and AM treatments on the P/Zn ratio in shoot (upper graphs) and roots (lower graphs) of bread (BE: Bezostaja, BD: BDME-10) and durum (KU: *Kunduru-1149*) wheat cultivars differing in Zn efficiency grown for 35 days in a Zn-deficient soil. The results represent means ± SE (vertical bars) of four independent replicates and were evaluated with two way ANOVA by using Sigma Stat software (***: 0.001<p, **: 0.01<p, *: 0.05<p, and ns: not significant)

nutritional status on the P/Zn ratio in roots was similar to that observed in shoots (*Figure 4.4*). Higher ratios were obtained in the roots of Zn deficient plants than in the Zn sufficient plants particularly at -Zn/high P treatment (*Figure 4.4*). The higher mycorrhizal activity at low P supply with or without Zn supply resulted an increase in the P/Zn ratio in roots (*Figure 4.4*). In the -Zn/high P treatment, mycorrhizal colonization had an increasing effect on the ratio of P/Zn (*Figure 4.4*).

Zinc and P uptake rates

The Zn and P uptake rates of wheat cultivars grown for 35 days at different Zn and P treatments with or without AM inoculation are given in *Figure 4.5*. The uptake rate was calculated by dividing the total amount of Zn or P in whole plants to root dry weight ([shoot content + root content] / root dry weight). As in concentration, Zn uptake rates also showed that under low P supply the wheat cultivars tended to take up more Zn at both -Zn and +Zn (*Figure 4.5*). Although all cultivars supplied with sufficient Zn did not show any tendency towards a relationship between Zn uptake rate and Zn efficiency, these cultivars under deficient supply of Zn had, in both P levels and with or without mycorrhizal inoculation (*Figure 4.5*). Besides, the mycorrhizal plants not supplied with Zn as in Zn concentration in roots had less uptake rates than plants grown without mycorhizae (*Figure 4.5*).

4.5. Discussion

Although, in many reports, the positive effect of mycorrhizal colonization on the plant growth has been noted, the presence of mycorrhizal infection had either no or a negative effect on shoot and root growth of the bread and durum wheat cultivars used in this study. This negative response was distinct in the plants grown at -Zn/high P treatment after 35 days growth in

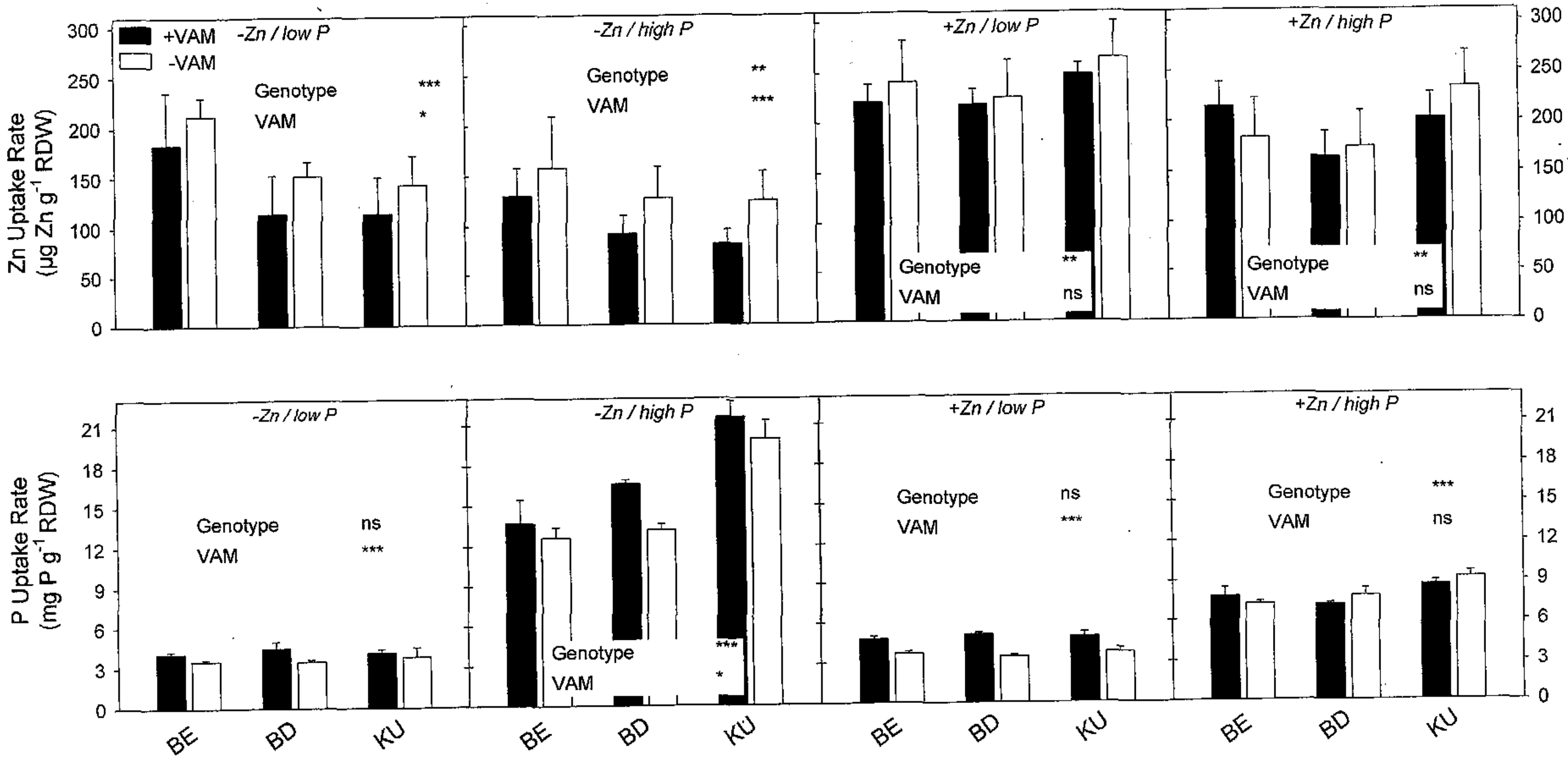


Figure 4.5. Effect of different Zn, P, and AM treatments on the uptake rates of Zn and P of bread (BE: Bezostaja, BD: BDME-10) and durum (KU: Kunduru-1149) wheat cultivars differing in Zn efficiency grown for 35 days in a Zn-deficient soil. The results represent means ± SE (vertical bars) of four independent replicates and were evaluated with two way ANOVA by using Sigma Stat software (***: 0.001<p, **: 0.01<p, *: 0.05<p, and ns: not significant)

an extremely Zn deficient soil (*Figure 4.1*). However, this finding is in accordance with the results obtained for wheat grown in sand, calcined clay, and loess soil (Hawkins, 2000). In that study, using three growth medium mentioned above, either no or negative effect of the presence of mycorrhizal colonization on the plant growth was shown. This lack of growth stimulation or hindered growth of the wheat plants in the presence of mycorrhizal infection may be attributed to the sensitivity of wheat to the changes in carbohydrate metabolism, particularly in the soils with low Zn availability (Rengel, 1995). Another clear observation at -Zn/high P treatment was higher Zn deficiency stress than -Zn/low P where plants showed no symptoms on leaf such as necrotic-patches (*Table 4.4*), but only small reduction in shoot growth (*Figure 4.1*). This effect could be explained by dilution of the accumulated Zn in shoot of the plants supplied with high P causing higher growth rates (*Figure 4.2*). In a recent study by Huang and his colleagues (2000), Zn deficiency was presented as a responsible factor for up-ragulated expression of high-affinity phosphate transporter genes in barley roots. In the light of this recent finding, the higher P concentrations in shoot and roots of Zn-deficient wheat genotypes, particularly at -Zn/high P treatment could be attributed to up-regulated expression of high-affinity phosphate transporter genes in wheat roots as in barley.

Irrespective of genotypical differences, the more severe leaf symptoms of Zn deficiency were observed in the mycorrhizal plants at -Zn/high P treatment compared to the non-mycorrhizal plants (*Table 4.4*). Since the presence of mycorrhizal colonization also represents a demand for carbohydrates, it may be thought that stress factors at -Zn treatment causes very important decreases in Zn-dependent enzymes an consequently decrease carbohydrate metabolism (Cakmak, 1988), plants showed more severe deficiency symptoms. Based on the severity of visual deficiency symptoms, susceptibility of wheat genotypes to Zn deficiency grown with high P and at both mycorrhizal treatment declined in the order Kunduru-

1149 > BDME-10 > Bezostaja (*Table 4.4*). These performances of genotypes are also in well-accordance to their reported order of Zn efficiency in literature (Cakmak et al., 1998) and results given in *Chapter 3*.

After 35 days of growth in Zn-deficient soil, the wheat cultivars responded to low P supply with a higher mycorrhizal colonization rates than the plants supplied with high P, irrespective of Zn supply (*Table 4.3*). Furthermore, at both P supplies the plants supplied with sufficient Zn were able to colonize the roots to higher degree (*Table 4.3*). These lower infection rates under Zn deficiency may also be related to the disturbed carbohydrate metabolism (Cakmak, 1988) and lower photosynthesis rate (Ajay & Rathore, 1995; Fischer et al., 1997).

Enhanced P uptake is a well-known phenomena in mycorrhizal plants (Bowen et al., 1975; Sanders et al., 1975; Abbott & Robson, 1977). The results obtained in this study (*Figure 4.3*), using two bread and one durum wheat genotypes, are also in good-agreement with the studies mentioned above. Particularly at low supply of P, the positive effect of AM on P uptake were more conspicuous. When the mycorrhizal colonization rates in roots (*Table 4.3*) and P concentrations in shoot and roots (*Figure 4.3*) are taken into account together, the correlation between mycorrhizal infection rate and P uptake can be observed. In particular, at high P treatment where Zn deficiency was stronger than lower P supply (*Table 4.4; Figure 4.1*), Zn deficiency increased the tissue concentration of P in shoot and also in roots (*Figure 4.3*). This enhanced P uptake may be attributed to up-regulated expression of high-affinity P transporter as shown in barley roots (Huang et al., 2000).

In general, Zn supply increased the tissue Zn concentration in shoot and roots, but especially in shoot (*Figure 4.2*). Among the plants supplied with sufficient Zn, the plants at low P treatment contained more Zn per shoot dry weight than the plants grown with high P (*Figure 4.3*). This lower Zn concentration at high P level could be explained by the phenomena named "dilution effect" which is a cause of increased growth of the plants (Marschner, 1995). In contrast, to some of the earlier findings (Gnekow & Marschner, 1989; Kothari et al., 1990; Li et al., 1991), the presence of mycorrhizal infection did not positively affect the uptake of Zn (*Figure 4.2 and 4.5*). In the plants supplied with no Zn, the AM even reduced the Zn uptake (*Figure 4.2 and 4.5*). Using chickpea, Weber and his colleagues (1993) also did not see any positive effect of mycorrhiza on Zn concentrations in shoot. They attributed this decrease in Zn concentration to the dilution of Zn in shoot tissue due to increased growth of AM-inoculated plants. This hindered Zn uptake by the mycorrhizal plants could also be explained by the disturbed energy metabolism in plants.

Unexpectedly, very high Zn concentrations were measured in the roots of wheat cultivars compared to the shoot values, even in the plants grown without Zn supply (*Figure 4.2*). Extremely high Zn accumulation in the roots under Zn deficiency in a Zn-deficient soil raises some questions as why/how/where the plants can accumulate so much Zn in the roots. In all genotypes supplied with 5 mg Zn kg^{-1} soil, Zn concentrations in the roots (*Figure 4.2*) and particularly Zn uptake rates did not differ from each other. However, among the plants grown under Zn deficiency, interestingly these values changed from one genotype to other and fitted to their susceptibilities to Zn deficiency, based on the severity of visual Zn deficiency symptoms and decreases in shoot dry matter production: Kunduru-1149 > BDME-10 > Bezostaja-1. In such a way that while the bread wheat cv. Bezostaja-1, the most Zn efficient, had the highest Zn concentration in roots (*Figure 4.2*) and uptake rates (*Figure 4.5*), the most susceptible one, durum wheat cv. Kunduru-1149, showed the lowest values for both. As a first impression, it may be thought that the contamination of Zn with soil particles stuck on roots is the main reason for high Zn contents per root dry weight (*Figure 4.2*) as shown in barley for Fe (Strasser, 2000). On the other hand, up to 2.5-fold higher Zn concentrations in the roots (200 mg kg^{-1} root dry wt.) under Zn deficiency than the total Zn content in the soil (78-85 mg kg^{-1} soil) does not allow such an interpretation. In contrast to the differences in Zn concentration (*Figure 4.2*) and Zn uptake rates (*Figure 4.5*) of the bread and durum wheat cultivars used in this study, the bread wheat cultivars do not differ in either PS release (*Chapter 6*; Erenoglu et al., 1996), in Zn uptake in nutrient solution (*Chapter 7*; Cakmak et al., 1998; Erenoglu et al., 1999), in remobilization of Zn from older leaves to younger growing parts (*Chapters 8* and *9*; Erenoglu et al., 2001a, b) or in mycorrhizal colonization (*Table 4.3*). The Zn concentrations in the shoot of the cultivars differed from each other at $0.001<p$ and $0.01<p$ for the plants grown -Zn/low P and -Zn/high P, respectively (*Figure 4.2*). Although the differences in the concentrations of Zn in the shoot tissue seemed to be well correlated with the Zn deficiency susceptibility of wheat genotypes used (*Figure 4.2*), in the field conditions they did not differ from each other. Similar Zn concentrations but dissimilar Zn contents per plant have been attributed to the dilution by growth (Marschner, 1995). This phenomena can be explained as follows: Under Zn deficiency, enhanced uptake and/or mobilization of Zn in efficient genotypes causes increases in shoot dry matter production which results in a corresponding dilution of Zn in tissues to similar concentrations as in the inefficient genotype. The total Zn uptake to 310 μg per pot (the most efficient bread wheat cv. Bezostaja at -Zn/low P treatment), despite 65 μg of DTPA-extractable Zn (plant available) per 650 g of the experimental soil, increases the possibility of the importance of the mobilization processes in rhizosphere. According to Uren (2001), the percentage of C used for secretions in total C fixed by soil grown plants is less than 1%. If it is thought that the rhizosphere processes, particularly the secretion of chelators, affect the mobilization and uptake of Zn, the lower Zn uptake by mycorrhizal plants in which C demand is higher than non-mycorrhizal plants under Zn deficiency may also be attributed to decreases in the secretion of chelators.

Increasing Zn deficiency enhanced the uptake and root-to-shoot transport of P (*Figure* 4.3 and 4.5), so much so that P/Zn ratios in the shoot and roots(*Figure* 4.4). The P/Zn ratio reached up to 900 in the plants supplied with no Zn and high P. (*Figure* 4.4). These P/Zn ratios at -Zn/high P treatment are much higher than the critical level, 150, given for leaves of grape grown in soil (Marschner & Schropp, 1977) and the most susceptible wheat cv. Kunduru-1149 had the highest P/Zn ratio. The plants supplied with low P did not increase much the P/Zn ratios and kept around critical level. The plants supplied with 5 mg Zn kg^{-1} soil were under critical level, irrespective of P supply.

In conclusion, the results show that, in a soil with extremely low available Zn, the presence of mycorrhizal colonization did not have a positive effect on Zn uptake in the bread and durum wheat genotypes used. Mycorrhizal colonization even had a negative effect on growth in some cases. However, P uptake was enhanced by mycorrhizal infection. The variable Zn uptake rates under Zn deficiency, irrespective of mycorrhizal infection, showed the role of rhizosphere processes in differential Zn mobilization by wheat cultivars differing in Zn efficiency in soil conditions needs to be clarified.

5. Phytosiderophore release in rye, triticale, bread wheat, and durum wheat genotypes differing in zinc efficiency

5.1. Abstract

One rye (*Secale cereale* cv. Aslim), one triticale (x *Triticosecale* Wittmark cv. Presto), three bread wheat (*Triticum aestivum* cvs. Dagdas-94, Gerek-79, and Bul 63-68-7), and one durum wheat (*Triticum durum* cv. Kiziltan-91) cultivars were grown under controlled environmental conditions in nutrient solution for 17 days to study the effect of varied supply of Zn (0 to 1 mmol m^{-3}) on PS release, Zn deficiency symptoms in shoots, and shoot and root dry matter production. Visual Zn deficiency symptoms, such as whitish-brown lesions on leaves, were observed rapidly and severely in durum wheat cv. Kunduru-1149 and bread wheat cv. Bul 63-68-7. Later on, among the bread wheats , Dagdas-94 and Gerek-79 were affected by Zn deficiency. Triticale cv. Presto and, particularly, rye cv. Aslim were the last genotypes which were influenced by Zn deficiency. In all genotypes supplied with adequate Zn, PS release was very low, around 0.5 µmol 48 $plants^{-1}$ $3h^{-1}$. However, under Zn deficiency, the PS release was enhanced in rye, triticale, and bread wheat genotypes. In contrast to them, the PS release in Zn deficient durum wheat cv. Kiziltan-91 remained at a very low levels. HPLC analysis of root exudates indicates that while 3-hydroxy-mugineic acid (HMA), 2′-deoxy-mugineic acid (DMA), and mugineic acid (MA) are released by the roots of rye and triticale, DMA is the main PS secreted by the roots of bread and durum wheat species.

The results demonstrate the enhanced synthesis and release of PS's under Zn deficiency. However, with the exception of the well-accordance between low PS release and low Zn efficiency of durum wheat, among the other genotypes PS release does not correlate well with Zn efficiency.

5.2. Introduction

Zinc deficiency is one of the well-recognized micronutrient deficiencies, in all over the world, in particular in calcareous soils of arid and semi-arid regions (Cakmak et al., 1996a, Takkar & Brennan, 1993). Soil or foliar application of Zn is a widely used agronomic practice for farmers to overcome Zn deficiency in plants. Selection and/or breeding of plant genotypes with higher resistance to Zn deficiency is an alternative and sustainable approach to over come this problem in plants.

Crop species are markedly different in their ability to adapt to Zn deficient soils. Among the cereal species, rice, sorghum, and maize are considered as Zn deficiency sensitive species, although barley, wheat, and rye are classified as less sensitive (Viets et al., 1954; Clark, 1990). Cakmak et al. (1997) showed that sensitivity of cereals to Zn deficiency declined in order : durum wheat > bread wheat > triticale > rye. They also suggested that the Zn efficiency trait of rye is transferable into the genome of wheat, because of the expression of high Zn efficiency in triticale, a wheat-rye hybrid. Also, the genotypes of a given species show large differences in sensitivity to Zn defficiency. Particularly in wheat, there are great genotypical differences in Zn efficiency. The reason for differential Zn efficiency of cereals, in particular of wheat genotypes was extensively studied, is still not well understood. For example, differences in root morphology (Dong et al., 1995), release of Zn-mobilizing phytosiderophores (Cakmak et al., 1994, 1996c; Walter et al., 1994), and Zn uptake capacity of roots (Cakmak et al., 1998; Rengel & Graham, 1996) were discussed as possible responsible mechanisms for expression of Zn efficiency.

Graminaceous species increase the synthesis and release of non-protein amino acids, called phytosiderophores to the rhizosphere, under deficiencies of Fe (Römheld, 1987; Takagi, 1976) and Zn (Cakmak et al., 1994; Walter et al. 1994; Zhang et al., 1989). The same types of phytosiderophores are released with the same diurnal rhythm under Zn and Fe deficiencies. Phytosiderophores are highly effective in complexing and mobilizing Fe and Zn in the rhizosphere (Takagi et al., 1988; Treeby et al., 1989) and within root apoplasm (Zhang et al., 1991).

In recent years, the role of phytosiderophores in Zn efficiency of wheat genotypes has been investigated by number of researchers (Cakmak et al., 1994, 1996c; Walter et al., 1994). In the first study by Cakmak et al. (1994) and in the study conducted by Walter et al. (1994) only one bread and one durum wheat genotypes were used. The results of these studies showed that differential susceptibility of the genotypes to Zn deficiency was well-related to the capacity of genotypes to release phytosiderophores from roots under Zn deficiency. In the second study by Cakmak et al. (1996c), they compared four bread and four durum wheat genotypes. According to the results of this study it was concluded that enhanced release of phytosiderophores under Zn deficiency is most likely causally involved in Zn efficiency of wheat genotypes.

Three types of method are used for qualitative and quantitative analyses of PS's in root exudates or other materials such as tissue extracts for PS's: i) Mobilization tests (Quantitative), ii) Thin Layer Chromatopgraphy (TLC; Qualitative and Semi-Quantitative), and iii) High Pressure Liquid Chromatography (HPLC; Qualitative and Quantitative). Since the mobilization tests are much faster than others, they are preferentially used very often by researchers (Fe-Mobilization - Takagi, 1976; Cu-Mobilization - Cakmak et al., 1996c; Cu-CAS - Shenker et al., 1995).

The aim of the present study was to determine release of phytosiderophores in rye, triticale, bread wheat, and durum wheat genotypes which differ in Zn efficiency in field and greenhouse experiments. In addition, a new ion exchange resin named Chelex 100 was tested to develop a special Zn-Mobilization test for Zn-PS studies.

5.3. Materials and methods

Plant growth

One rye (*Secale cereale* cv. Aslim), one triticale (x *Triticosecale* Wittmark cv. Presto), three bread wheat and (*Triticum aestivum* cvs. Dagdas-94, Gerek-79, and Bul 63-68-7), and one durum wheat (*Triticum durum* cv. Kiziltan-91) genotypes were grown in nutrient solution under controlled environmental conditions (light/dark regimes of 16/8 h at 24/20 °C, relative humidity 65-75%, and a photosynthetic photon flux of 350 μmol m^{-2} s^{-1} at plant height provided by Osram Sylvania cool white FR96 T12 tubes). The cultivars used in this study were selected based on their growth under field conditions in a severely Zn-deficient calcareous soil in Central Anatolia, Turkey. Among the cultivars, rye shows the highest efficiency and triticale follows it. Susceptibility of bread wheats to Zn deficiency grown on a Zn deficient calcareous soil decreases in the order Bul 63-68-7 > Dagdas-94 = Gerek-79. Durum wheat cv. Kiziltan-91 has the lowest Zn efficiency.

After germination in quartz sand moistened with saturated $CaSO_4$ solution, seedlings were transferred to 2.5-L plastic vessels containing the following continuously aerated nutrient solution: (in mol m^{-3}) 0.88 K_2SO_4, 2.0 $Ca(NO_3)_2$, 0.25 KH_2PO_4, 1 $MgSO_4$, 0.1 KCl, (in mmol m^{-3}) 100 FeEDTA, 1 H_3BO_3, 0.5 $MnSO_4$, 0.2 $CuSO_4$, and 0.02 $(NH_4)_6Mo_7O_{24}$. Zinc was supplied as $ZnSO_4$ at concentrations of 0 mmol m^{-3} (for Zn deficient plants) and 1 mmol m^{-3} (for Zn sufficient plants).

Collection of root exudates

On days 11, 13, 15, and 17, root exudates were collected for the measurement of release of PS's by roots. For the collection of root exudates, 2h after the onset of the light period, intact plants were removed from nutrient solution, and the roots were washed in deionized water. Thereafter, plants were placed in 500 ml aerated deionized water for 3h. Root exudates collected were treated with Micropur (Roth GmbH, Karlsruhe, Germany) to prevent microbial degradation of PS's, filtered through coarse filter paper, concentrated under vacuum to 20 mL and stored at -20 °C in deep-freezer until qualitative and quantitative analyses.

Testing of Chelex 100 resin for quantitative analysis of PS's

A new chelating ion exchange resin (Chelex 100, BIO RAD Laboratories) was used to develop a Zn-mobilization test and to compare Zn- and Fe-loaded resin. Phytosiderophore (HMA) standardized by HPLC and HEDTA were used in this study as Zn and Fe mobilizing chelators. Firstly, since Chelex 100 was available as Fe^{III}-loaded resin from the company, this Fe^{III}-loaded resin was used to test the effect of pH on Fe^{3+} mobilization by HEDTA. Twenty five grams of Fe^{III}-loaded Chelex 100 was equilibrated with 500 ml 10 mol m^{-3} MES (pH 4.5, 5.0, 5.5, 6.0, or 6.5). Then, 2 ml of FeIII-loaded Chelex 100 was mixed with the necessary amount of chelator (HEDTA) and the volume was completed to 10 mL. Thereafter, this suspension was shaken on a reciprocal shaker and then filtered. Further, Fe^{3+} mobilization capacities of HEDTA and HMA from FeIII-loaded Chelex 100 were compared. In this experiment, pH 5.5 which was found in the first experiment as the most useful pH, was used and the rest of the experimental procedure was the same as explained before. In the last part of the test, Fe^{3+} or Zn^{2+} mobilization abilities of HMA and HEDTA from Fe^{III}- or Zn^{II}-loaded resin were compared. The procedure for Fe^{3+} mobilization from Fe^{III}-loaded Chelex 100 was as in second step of this experiment. To obtain Zn-loaded Chelex 100 following procedure was followed: 25 g of Chelex 100 was washed in 100 ml 1 M HCl and thereafter, this suspension was transferred into a labor glass-tube and until obtaining drops with pH 5.5-6.0 (pH of de-ionized water) from the tap of the tube. Later on, the resin was treated with Zn in 50 mol m^{-3} $ZnSO_4$ by stirring for 15 minutes. After it, the suspension obtained was poured into glass-tube and washed with deionized water until obtaining Zn-free drops from the tap of the tube. At the end, Zn-loaded Chelex 100 was also transferred into 10 mol m^{-3} MES buffer (pH 5.5) and the same experimental procedure as explained for Fe^{3+} mobilization was followed to measure Zn^{2+} mobilization.

Copper (Cu) Mobilization Test

The amount of PS's in the root exudates was determined by measurement of Cu mobilized from a Cu-loaded resin (Chelite-N. 0.05-0.1 mm, Serva, Heidelberg, Germany). The Cu-Chilete-N complex was prepared as follows (Cakmak et al., 1996c). As a start step, 5 g Chelite-N was washed in 100 ml 1 N HCl and afterwards, this suspension was poured into a glass-tube and up to pH about 5.5-6 washed with deionized water. Thereafter, the Chelite-N was loaded with Cu in 50 mol m^{-3} $CuSO_4$ by stirring for 15 min. The suspension obtained was again transferred into glass-tube and washed with deionized water until obtaining Cu-free drops from the tap of the glass-tube. Then, the Cu-loaded resin was equilibrated with 500 mL of 10 mol m^{-3} MES buffer (pH 5.0) and used for Cu mobilization tests. Two mL of concentrated and in deep-freezer stored root exudates were added into 2 mL of Cu-loaded resin and 6 ml of deionized water mixture and shaken for 45 min. on a reciprocal shaker and then filtered. The concentration of Cu in the filtrate was measured by AAS, and the amount of PS's in the root exudates was calculated as mobilized Cu equivalents per 48 plants or per g root dry weight.

HPLC analysis of root exudates

For the qualitative analysis of PS's in root exudates by HPLC, concentrated root exudates were membrane-filtered (0.2 µm) and used directly for HPLC analysis as described by Mori et al. (1987).

5.4. Results

The amounts of mobilized Fe^{3+} by increasing amounts of HEDTA at a pH range between 4.5 and 6.5 from Fe-loaded Chelex 100 are plotted in *Figure 5.1*. Among all pH values, the range 5.5-6.0 caused higher Fe^{3+} mobilization compared to others, showing the most suitable pH range. For this reason, pH 5.5 was used for the next steps of Chelex 100 test.

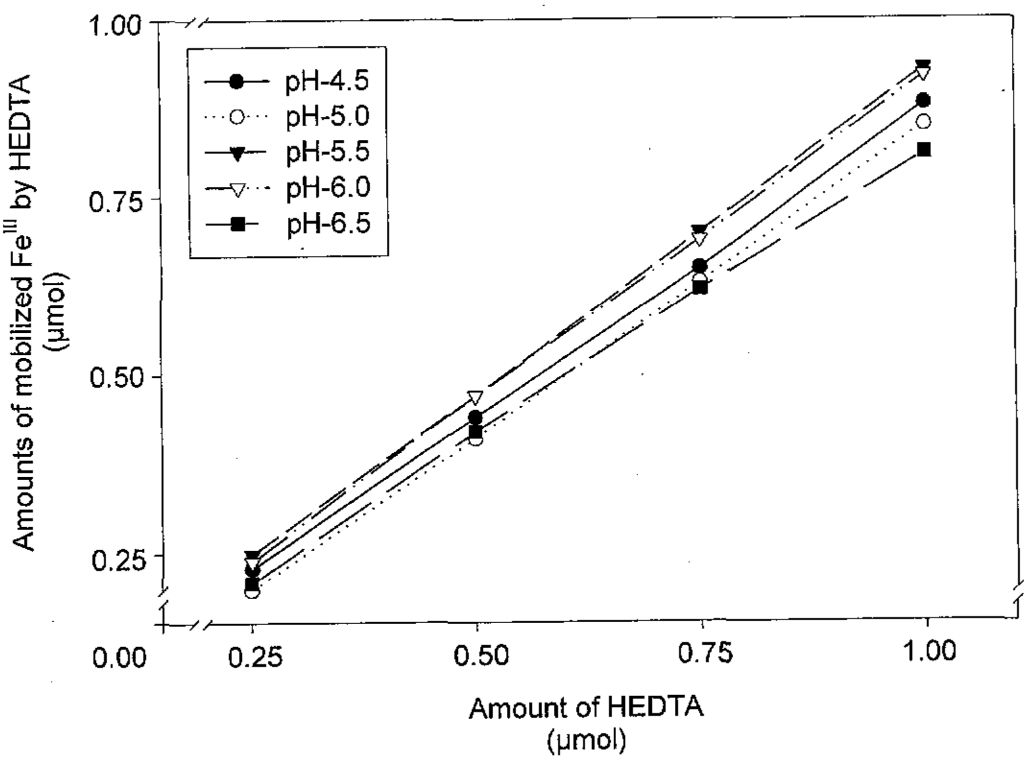


Figure 5.1. Effect of pH on Fe^{3+} mobilization by HEDTA from Fe^{III}-loaded Chelex 100 (Data represent means of three independent replications and SEs are not shown since they are too low)

Iron mobilization capacities of HEDTA and PS from Fe^{III}-loaded Chelex 100 are given in *Figure* 5.2. As seen in this figure, PS can mobilize more Fe^{3+} from Fe^{III}-loaded Chelex 100 resin than HEDTA. Up to 1 µmol chelating agent, there was more or less straight relation between amounts of chelator and capacity for Fe mobilization, particularly for PS.

The results of the experiment conducted to compare the Fe^{3+} and Zn^{2+} mobilization abilities of PS and HEDTA from Fe^{III}- or Zn^{II}-loaded resin indicate that PS can mobilize Fe^{3+} from Fe^{III}-loaded resin, but only very few amounts of Zn^{2+} from Zn^{II}-loaded resin (*Figure* 5.3). Obviously, in contrast to HEDTA, PS is not strong enough to mobilize Zn from Zn^{II}-loaded Chelex 100.

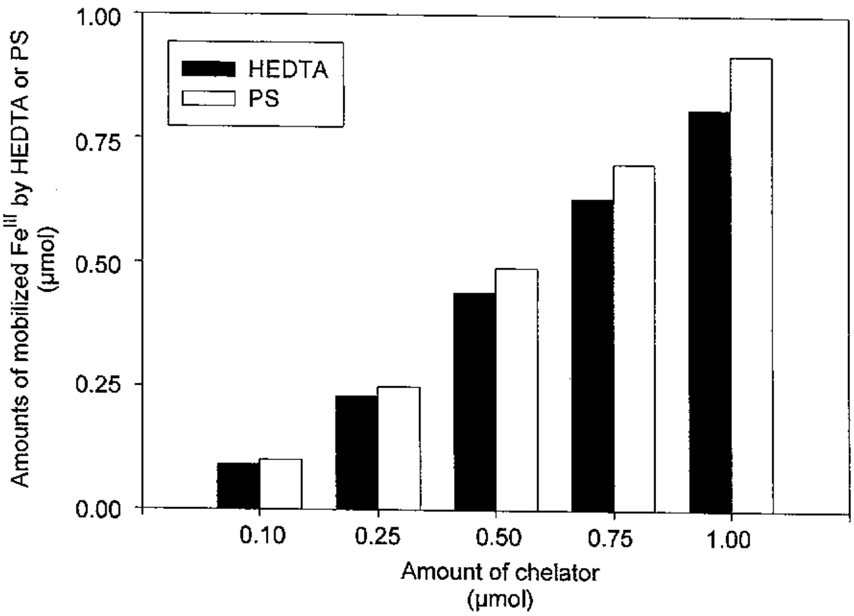


Figure 5.2. Fe^{3+} mobilization by PS or HEADTA from Fe^{III}-loaded Chelex 100 at pH 5.5 (Data represent means of three independent replications and SEs are not shown since they are too low)

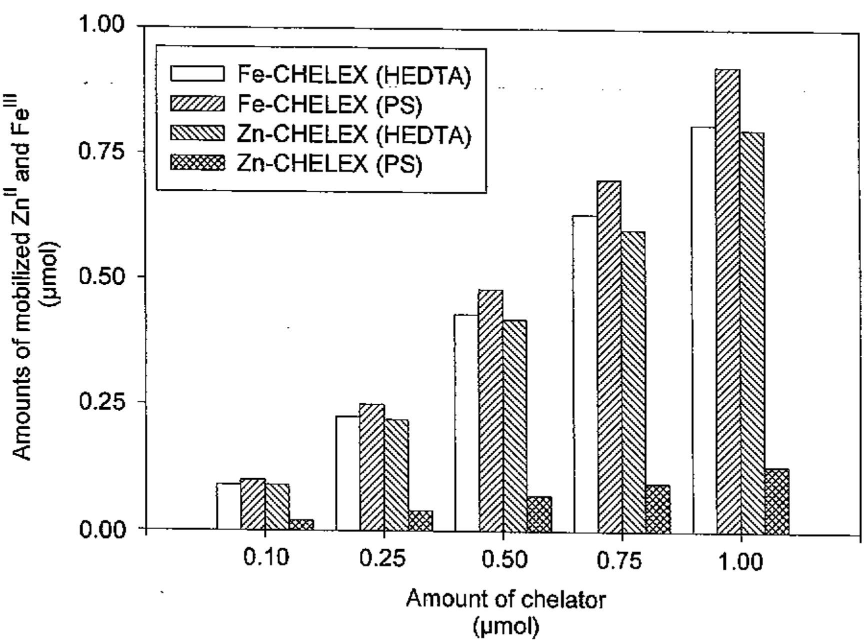


Figure 5.3. Fe^{3+} or Zn^{2+} mobilization by PS or HEDTA from Fe^{III}-loaded or Zn^{II}-loaded Chelex 100 at pH 5.5 (Data represent means of three independent replications and SEs are not shown since they are too low)

Under Zn deficiency, shoot dry matter production of genotypes decreased (*Table 5.1*). No distinct differences in shoot dry matter production of genotypes used without Zn application were detected. In contrast to shoot growth, root growth of all genotypes while in some genotypes increased, in others decreased but this depression in root growth was not as much as in shoot growth (*Table 5.1*).

Shoot/root dry weight ratios were lower in Zn deficient plants than in Zn sufficient plants (*Table 5.1*). There was also no relationship between shoot/root ratios and susceptibility of these genotypes to Zn deficiency when grown in a Zn deficient soil.

The second characteristic reaction of plants to Zn deficiency, necrotic patches on leaf blade, appeared firstly in the durum wheat cv. Kiziltan-91 and the bread wheat cv. Bul 63-68-7. Later on, the other bread wheat cvs. Dagdas-94 and Gerek-79 showed this typical symptom of Zn deficiency. In the triticale cv. Presto and particularly in the rye cv. Aslim, this reaction was observed very late. However, differences in sensitivity to Zn deficiency among the genotypes were less distinct in a nutrient solution than in a Zn-deficient soil grown in greenhouse condition (Cakmak et al., 1998).

In all genotypes supplied with adequate Zn, release of PS's was very low and around 1 μmol 48 plants^{-1} 3h^{-1} over the 17 days growth (*Figure 5.4*). Also in the Zn deficient durum wheat, amount of PS's release was very low and did not exceed 2.6 μmol 48 plants^{-1} 3h^{-1}. In contrary, in Zn deficient rye, triticale, and bread wheats, amount of PS's release was markedly increased, even in the Zn inefficient bread wheat cv. Bul 63-68-7. Obviously, there was a poor relationship between the amount of PS's release and the susceptibility of genotypes with the exception of durum wheat, to Zn deficiency (*Figure 5.4*). This behavior of species and cultivars of a species did not change when the PS release was expressed on a root dry weight basis (*Figure 5.5*).

Table 5.1. Shoot and root dry weight and shoot/root dry weight ratios of various cereal genotypes grown with (+Zn=1 μ*M*) and without (-Zn) Zn supply in nutrient solution for 9 and 13 days. Data represents means ± SE of three independent replicates

	Dry weight, mg plant^{-1}											
	9 days						13 days					
	Shoot		Root		Shoot/Root		Shoot		Root		Shoot/Root	
Genotypes	+Zn	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn	-Zn
S. cereale												
Aslim	45 ± 3	32 ± 6	11 ± 2	15 ± 3	4.1	2.1	126 ± 7	61 ± 5	30 ± 7	42 ± 1	4.2	1.5
xTriticosecale												
Presto	57 ± 4	46 ± 3	22 ± 2	26 ± 5	2.6	1.8	141 ± 2	81 ± 5	52 ±8	59 ± 3	2.8	1.4
T. aestivum												
Dagdas-94	77 ± 3	49 ± 6	36 ± 2	28 ± 4	2.1	1.8	162 ±	80 ± 7	57 ± 1	59 ± 3	2.8	1.4
Gerek-79	50 ± 2	36 ± 3	27 ± 2	23 ±3	1.9	1.6	131 ±	51 ± 6	48 ± 5	37 ± 2	2.7	1.4
Bul 63-68-7	48 ± 7	35 ± 3	20 ± 1	19 ± 3	2.4	1.8	115 ± 2	56 ± 2	46 ± 3	33 ± 3	2.5	1.7
T. durum												
Kiziltan-91	50 ± 5	41 ± 3	25 ± 4	22 ± 1	2.0	1.9	119 ± 3	75 ± 7	46 ± 4	37 ± 1	2.6	2.0

90

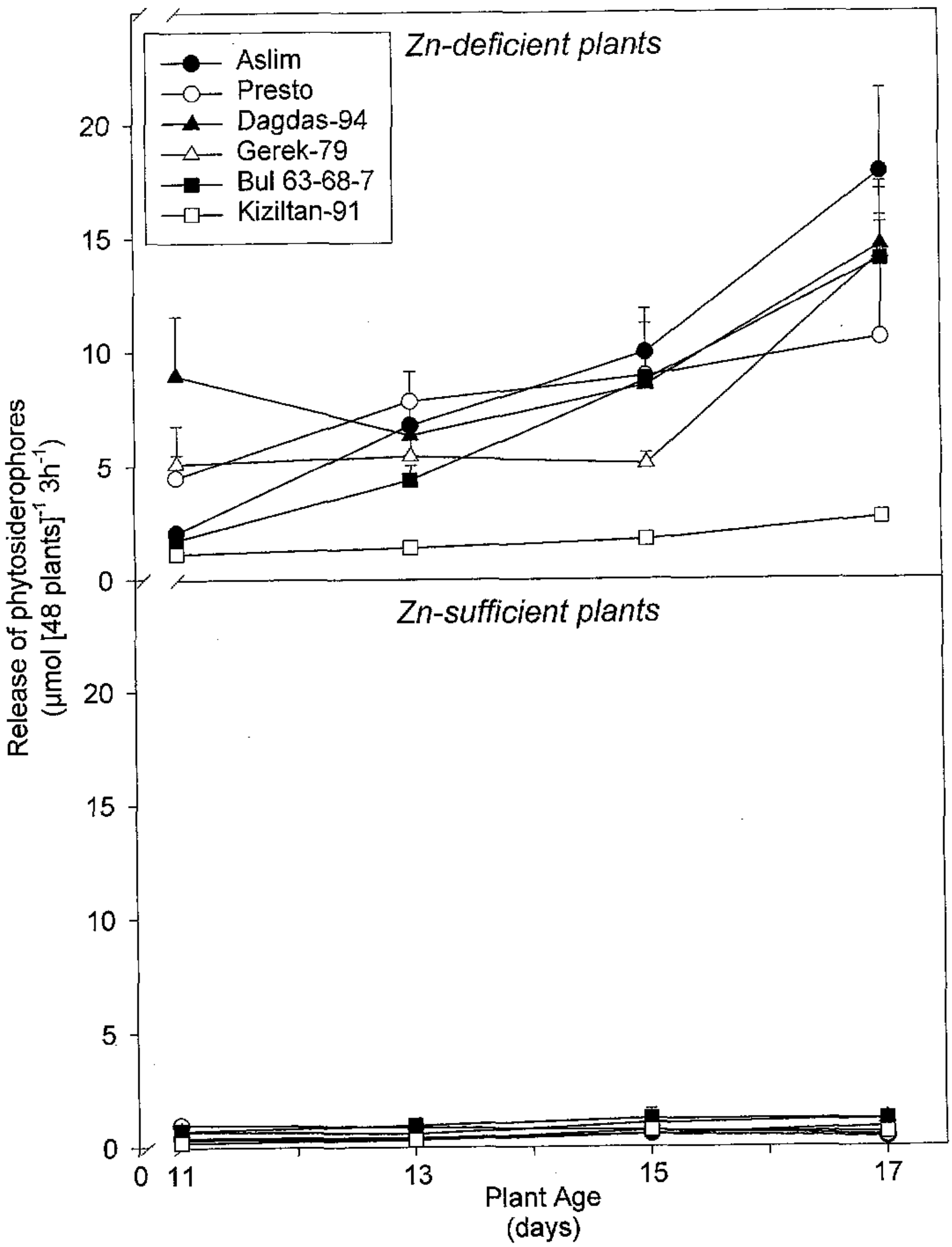


Figure 5.4. Effect of Zn nutritional status on the release of phytosiderophore from roots of various cereal genotypes differing in Zn efficiency during 17 days growth in nutrient solution. Results represent means ± SE of three independent replicates

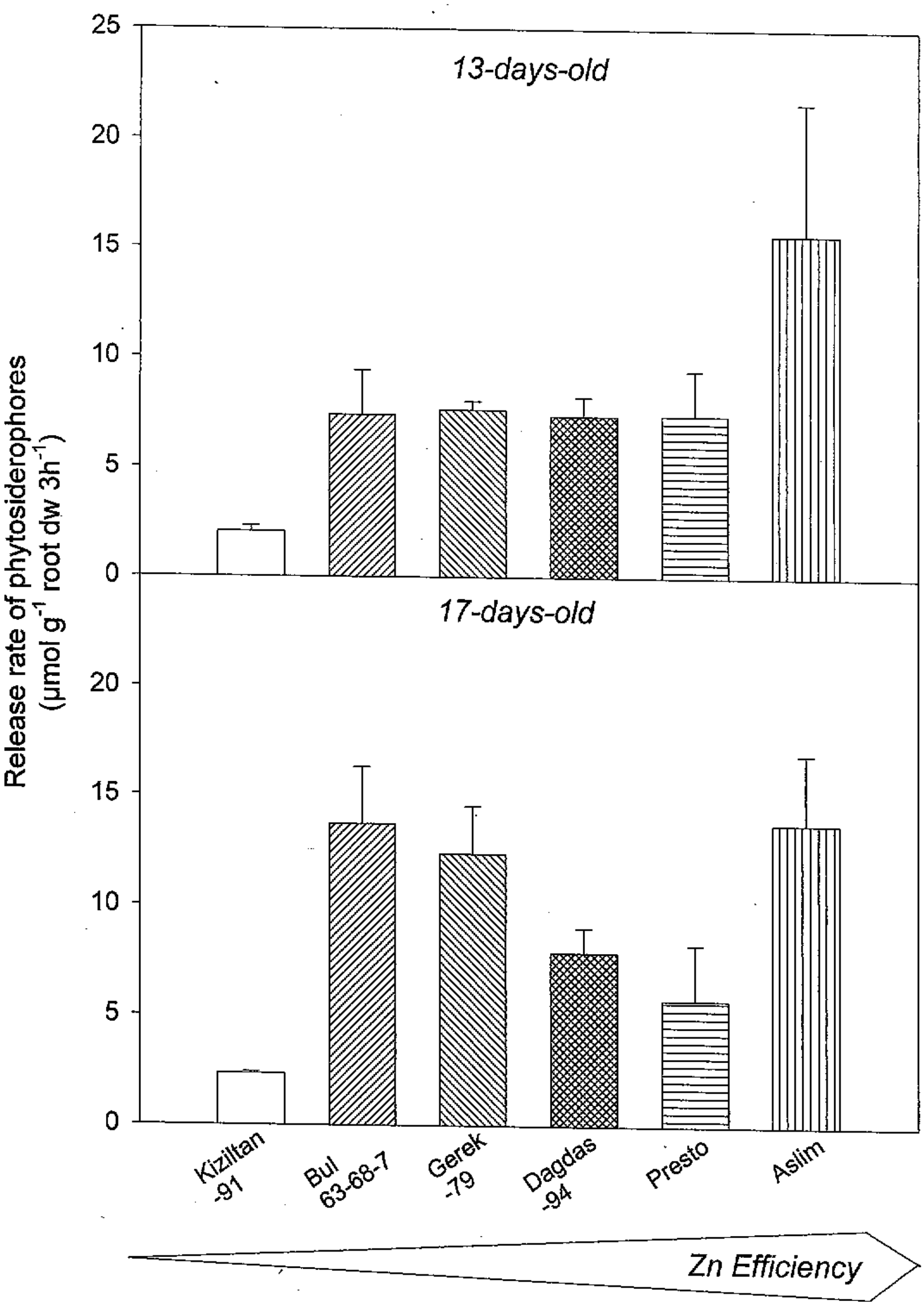


Figure 5.5. Relationship between phytosiderophore release and Zn efficiency of wheat genotypes grown for 17 days in nutrient solution without Zn supply. Results represent means ± SE of three independent replicates

HPLC chromatograms of root exudates from 17-days-old Zn deficient plants are presented in *Figure 5.6*. As seen in this figure, while rye and triticale released HMA, MA, and DMA together, wheat cultivars released only DMA.

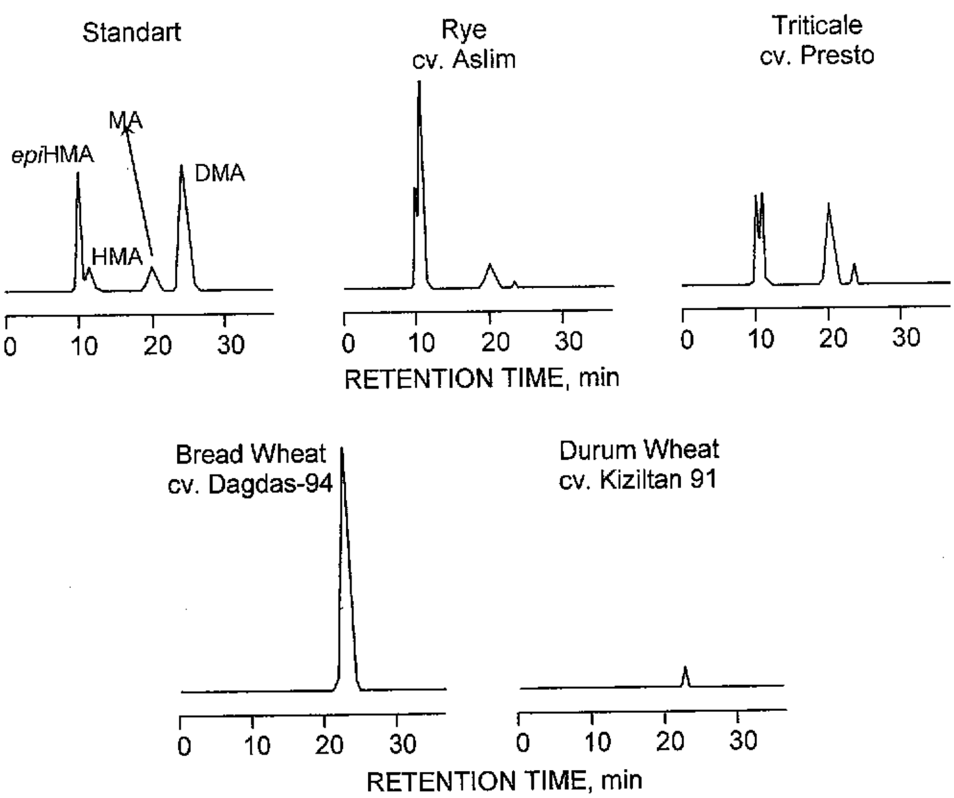


Figure 5.6. HPLC chromatograms of root exudates from 17-days-old Zn deficient plants

5.5 Discussion

Among the PS analysis techniques, mobilization tests are known to be fast and accurate. In this study a new resin, named as Chelex 100 was tested as a specific mobilization test for Zn-PS studies and the results are given in *Figure 5.1, 5.2, and 5.3*. Although PS's are able to mobilize Fe^{3+} from Fe^{III}-loaded Chelex 100, Zn^{2+} could not be mobilized from Zn^{II}-loaded Chelex-100 by PS's.

However, HEDTA can mobilize Zn^{2+} from Zn^{II}-loaded Chelex 100, showing that the bond between resin and Zn^{2+} is too strong for being extracted by PS's. According to Nomoto et al. (1987), the log stability constants of mugineic acid with Zn^{II}, Cu^{II}, and Fe^{III} are 10.7, 18.3, and 18.1, respectively. This lower log stability constant of PS with Zn^{II} can be the reason for the low Zn mobilization ability of PS's from Zn^{II}-loaded Chelex 100. In the light of all these findings, another mobilization method named "Cu-Mobilization" used by Cakmak et al. (1996c) was selected to analyze the root exudates collected.

There were very well accordance between the Zn deficiency symptoms, such as necrotic patches on leaf blade, observed on the genotypes and those reported in the literature (Cakmak et al., 1996a, b, 1998; Rengel and Graham, 1995a) and those observed in *Chapter 3* of this PhD thesis. Differences among genotypes in the development time and severity of Zn deficiency symptoms were also observed. However, possibly, because of lacking of rhizosphere, so thus Zn, these differences were not so considerable as in soil. Durum wheat cv. Kiziltan-91 was highly sensitive to Zn deficiency as observed in a Zn deficient soil under field or greenhouse conditions (Cakmak et al., 1996a, 1998; Graham et al., 1992).

The results show that enhanced release of PS's from roots of graminaceous species is not a specific response to Fe deficiency, also happens under Zn deficiency (*Figure 5.4* and *5.5*). This result is in full agreement with the findings of Zhang et al. (1989), Cakmak et al., (1994; 1996c), Rengel et al. (1998), and Walter et al. (1994) using different wheat genotypes. In contrast to these concordant results, Gries et al. (1995) using only one barley genotype and Pedler et al. (2000) using one bread and one durum wheat genotypes could not find any increase in release of PS's under low Zn supply.

In the Zn deficient plants with the exception of durum wheat cv. Kiziltan-91, the amounts of PS release were up to 35-fold higher than in Zn sufficient plants (*Figure 5.4*). In durum wheat cv. Kiziltan-91, it was around 5-

fold. Durum wheats are well-known as particularly Zn deficient (Cakmak et al., 1996a, 1998; Graham et al., 1992). The results presented here are in well-agreement with the earlier studies in which the lower capacity of durum wheats to synthesize and release PS's was discussed as one of the possible reasons for higher sensitivity of durum wheats to Zn deficiency (Cakmak et al., 1994, 1996c; Walter et al., 1994). Nevertheless the results of present study indicate that the different Zn efficiency of rye, triticale, and bread wheat genotypes can not be attributed to the differences in their PS release capacities.

If the qualitative analysis of different cereal species is considered, it can be seen that the triticale cv. Presto released DMA, MA, HMA and *epi*HMA similar to the rye cv. Aslim, suggesting that the characteristics of rye are dominant in triticale compared to bread wheat (*Figure 5.6*). This also fits to the suggestions done by Cakmak et al. (1997) that the Zn efficiency trait of rye is transferable into the genome of wheat.

Seemingly, some other mechanisms in the rhizosphere or within plants are effective in expression of Zn efficiency in cereals. As suggested by Dong et al. (1995, 1996), the differential root morphology might be a contributing factor to Zn efficiency. As an additional mechanism determining Zn efficiency in cereals, different Zn uptake capacities of roots and translocation of Zn to shoot can be proposed (*Chapter 7*; Cakmak et al., 1996b; Rengel & Graham, 1995b, 1996).

Additionally, differences in internal utilization or mobility of Zn can be relevant factors of differential Zn efficiency. Differential Zn efficiency among and within cereal species is better related to the total amount of Zn per shoot, but not to the Zn concentration in the shoot dry matter (Cakmak et al., 1997, 1998). However, Cakmak et al. (1998) showed that the Zn-efficient rye and bread wheat genotypes contain greater activity of Zn-containing superoxide dismutase in their leaves than the Zn-inefficient bread and durum wheat cultivars. These results suggest higher amounts of physiologically active Zn in leaf tissue of efficient genotypes. Rengel (1995) also showed higher carbonic anhydrase activity in a Zn efficient bread wheat compared to a Zn inefficient bread wheat cultivar.

The results presented in this chapter of this PhD thesis indicate that there is not always a well correlation between PS release by roots and Zn efficiency of cereal genotypes. Only higher sensitivity of durum wheats to Zn deficiency can be attributed to less PS release by the plants of this species. Some other mechanisms such as differential Zn uptake by roots and root-to-shoot transport of Zn, and differences in remobilization in plants and utilization at the cellular level might be effective in expression of Zn efficiency.

6. Phytosiderophore release does not relate well with zinc efficiency in different bread wheat genotypes

6.1. Abstract

Using six bread wheat genotypes (*Triticum aestivum* L. cvs. Dagdas-94, Gerek-79, BDME-10, SBVD 1-21, SBVD 2-22, and Partizanka Niska) and one durum wheat genotype (*Triticum durum* L. cv. Kunduru-1149) experiments were carried out to study the relationship between the rate of phytosiderophore release and susceptibility of genotypes to zinc (Zn) deficiency during 15 days of growth in nutrient solution with (1 µM Zn) and without Zn supply. Among the genotypes, Dagdas-94 and Gerek-79 are Zn efficient, while the others are highly susceptible to Zn deficiency, when grown on severely Zn deficient calcareous soils in Turkey. Similar to the field observations, visual Zn deficiency symptoms, such as whitish-brown lesions on leaf blades occurred first and severely in durum wheat Kunduru-1149 and bread wheat cvs. Partizanka Niska, BDME-10, SBVD 1-21, and SBVD 2-22. Visual Zn deficiency symptoms were less severe in the bread wheat cvs. Gerek-79 and particularly Dagdas-94. These genotypic differences in susceptibility to Zn deficiency were not related to the concentrations of Zn in shoots or roots. All bread wheat genotypes contained similar Zn concentration in the dry matter. In all genotypes supplied adequately with Zn, the rate of phytosiderophore release was very low and did not exceed 0.5 µmol 48 plants^{-1} 3h^{-1}. However, under Zn deficiency the release of phytosiderophores increased in all bread wheat genotypes, but not in the durum wheat genotype. The corresponding rates of phytosiderophore release in Zn deficient durum wheat genotype was 1.2 µmol and in Zn deficient bread wheat genotypes ranged between 8.6 µmol for Partizanka

Niska to 17.4 μmol for SBVD 2-22. In Dagdas-94, the most Zn efficient genotype, the highest rate of phytosiderophore release was 14.8 μmol. The results indicate that the release rate of phytosiderophores does not relate well with the susceptibility of bread wheat genotypes to Zn deficiency. Root uptake and root-to shoot transport of Zn and particularly internal utilization of Zn may be more important mechanisms involved in expression of Zn deficiency in bread wheat genotypes than release of phytosiderophores.

6.2. Introduction

Plant species as well as cultivars within a given species, e.g., wheat, differ markedly in their susceptibility to Zn deficiency (Graham and Rengel, 1993; Rengel and Graham, 1995a; Cakmak et al., 1996a). Variations in sensitivity to Zn deficiency in wheat are often related to differences in Zn uptake by roots or Zn accumulation per shoot, but not the concentration of Zn per unit dry weight (Graham et al., 1992; Rengel and Graham, 1995b; Cakmak et al., 1996b). The reason for genotypic differences in Zn uptake or accumulation in plants is still not clear (Graham and Rengel, 1993).

Enhanced release of phytosiderophores of the mugineic acid family from roots of graminaceous species is a well-known phenomena under Fe deficiency, but also under Zn deficiency, and suggested to be involved in mobilization and uptake of Zn from soil (Zhang et al., 1989, 1991a, b; Marschner, 1993). Under Fe deficiency, roots of graminaceous species release high amounts of phytosiderophores, and this release is closely related with differences in resistance to Fe deficiency of graminaceous species or cultivars of a given species (Takagi et al., 1984; Jolley and Brown, 1989; Marschner and Römheld, 1994). Similarly, differences in Zn efficiency between durum and bread wheat genotypes were also related t differences in root release of phytosiderophores (Cakmak et al., 1994, 1996c, Walter et al., 1994). Moreover, enhanced synthesis and release of Zn-mobolizing phytosiderophores from roots have been suggested t be a releavt mechanism for wild grasses in adaptation to severely Zn deficient calcareous soils (Cakmak et al., 1996d).

The bread wheat cultivars used in the study of Cakmak et al. (1996c) were either highly or moderately Zn efficient and none of them was highly susceptible to Zn deficiency like durum wheat cultivars. Therefore, experiments were conducted to measure the release rate of phytosiderophores in bread wheat genotypes showing large differences in severity of visual Zn deficiency symptoms when grown on Zn deficient soils in Turkey. Among the bread wheat genotypes studied, Dagdas-94 and Gerek-79 are Zn efficient and develop slight visual Zn deficiency symptoms on severely Zn deficient calcareous soils, while SBVD 2-22, SBVD 1-21, Partizanka Niska, and particulary BDME-10, are highly sensitive to Zn deficiency (Cakmak et al., 1996a). A Zn inefficient durum wheat genotype (Kunduru-1149) was also used in the experiments to evaluate better the release rates of pfytosiderophores among bread wheat genotypes.

6.3. Materials and methods

Plant growth

Six bread wheat cultivars (Triticum aestivum L. cvs. Dagdas-94, Gerek-79, SBVD 1-21, SBVD 2-22, BDME-10, and Partizanka Niska) and one durum wheat cultivar (Triticum durum L. cv. Kunduru-1149) were grown for 15 days in nutrient solution under controlled environmental conditions (light/dark periods of 16/8 h at 24/20 °C, relative humidity of 65-75% and a photosynthetic photon flux of 420 μmol $(m^2)^{-1}$ s^{-1} at plant height provided by Osram Sylvania cool white FR96 T12 tubes). Seeds were germinated in quartz sand moistened with saturated $CaSO_4$ solution. After five days, the seedlings were transferred to 2.5-L plastic vessels with continuously aerated nutrient solution (composition of nutrient solution is given in Chapter 5). For control plants (Zn sufficient plants), Zn was supplied as $ZnSO_4$ at a concentration of 1 mmol m^{-3}; no Zn was added to the nutrient solution for Zn deficient plants. The water used for nutrient solution was double-distilled.

On days 9, 11, 13, and 15, root exudates were collected for the measurement of phytosiderophore release from roots. For the collection of root exudates, intact plants were removed from nutrient solution 2 h after the onset of the light period, and the roots immersed 2 times for one minute in deionized water. Thereafter, plants were placed in 500 mL aerated double-distilled water for 3 h. Collected root exudates were treated with Micropur at 10 mg L^{-1} (Roth GmbH, Karlsruhe, Germany) to prevent microbial degradation of phytosiderophores during preparation for analysis. Then, root exudates were filtered and concentrated under vacuum to 20 mL for measurement of phytosiderophores by a Cu-mobilization (as described in *Chapter 5*).

The concentration of Zn in plants was measured by ashing samples at 550 °C, dissolving the ash in 3.3% HCl and measurement by atomic absorption spectrometry (AAS).

6.4. Results

Similar to the field and the greenhouse experiments in Zn deficient soils, visual Zn deficiency symptoms such as inhibition in shoot elongation and development of necrotic patches appeared first and more severe in the durum wheat cultivar Kunduru-1149, and thereafter in the bread wheat cultivars Partizanka Niska, BDME-10, SBVD 1-21, and SBVD 2-22 (*Table 6.1*). The bread wheat cultivars Gerek-79 and particularly Dagdas-94 were less chlorotic and necrotic.

Decreases in dry matter production by Zn deficiency were evident after 11 days of growth in nutrient solution and became more severe after 15 days (*Table 6.2*). Kunduru-1149 and Partizanka Niska were the genotypes showing highest Zn deficiency-induced decrease in dry matter production. After 11 days, Zn deficiency-induced decrease in dry matter production was evident only in shoots but not roots (*Table 6.2*). These differences in response of shoots and roots became more distinct after 15 days growth in nutrient solution.

The differences in sensitivity of genotypes to Zn deficiency were not related to the concentration or content of Zn in seeds (*Table 6.3*). The Zn concentrations per unit dry weight or per seed did not differ greatly among the genotypes.

Table 6.1. Visual Zn deficiency symptoms (necrotic patches on leaf blades)[a] of wheat genotypes grown in Zn deficient calcareous soils in the field and in nutrient solution

Genotype	Field[b] (Stem elongation stage)	Plants grown in Nutrient solution (15 days old)
Triticum aestivum		
Dagdas-94	1	2
Gerek-79	1	2-3
SBVD 1-21	3	3-4
SBVD 2-22	3	3
BDME-10	4	4
Partizanka Niska	4	4
Triticum durum		
Kunduru-1149	4	4

[a]Visual Zn deficiency symptoms: 1=slight; 2=mild; 3=severe; 4=very severe.

[b]See Cakmak et al. (1996a) for more detail on growth of Dagdas-94, Gerek-79, BDME-10, and Kunduru-1149 on severely Zn-deficient calcareous soils in Turkey.

Table 6.2. Dry matter production of different wheat genotypes grown with (+Zn= 1 mmol m^{-3}) and without (-Zn) Zn supply in nutrient solution for 11 and 15 days old

	Dry matter production (g dry weight [48 plants]$^{-1}$)							
	11 days old				15 days old			
	Shoots		Roots		Shoots		Roots	
Genotype	+Zn	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn	-Zn
Triticum aestivum								
Dagdas-94	2.5	1.9	1.0	1.3	6.4	3.0	2.0	2.3
Gerek-79	2.4	1.8	1.2	1.2	6.3	2.5	1.8	1.8
SBVD 1-21	2.8	1.7	1.1	1.3	7.6	3.1	2.1	2.2
SBVD 2-22	2.6	1.7	1.0	1.4	6.7	2.7	1.8	1.9
BDME-10	2.6	1.8	1.1	1.0	6.9	3.0	2.2	1.7
Partizanka Niska	3.0	1.5	1.2	1.0	7.4	2.7	2.2	1.4
Triticum durum								
Kunduru-1149	3.0	1.5	1.1	1.1	6.3	2.1	1.8	1.4
LSD (0.05)	0.2	0.1	0.1	0.1	0.5	0.2	0.2	0.1

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Concentrations of Zn in the shoots and roots were much higher in plants adequately supplied with Zn than in plants without Zn supply (*Table 6.4*). Under Zn deficiency, shoot concentrations of Zn were similar in all bread cultivars and varied between 4.3 and 5 μg g^{-1} dry weight. In contrast to shoots, root concentrations of Zn were more variable and ranged between 6.7 and 8.5 μg g^{-1} dry weight (*Table 6.4*).

Table 6.3. Concentration and content of Zn in seeds used in the experiment. Data are means (±SD) of three replicates

	Seed Zn	
Genotypes	μg Zn g^{-1} DW	ng Zn $seed^{-1}$
Triticum aestivum		
Dagdas-94	10.2 ± 0.4	468 ± 10
Gerek-79	9.7 ± 0.2	338 ± 7
SBVD 1-21	12.6 ± 0.1	533 ± 11
SBVD 2-22	10.0 ± 2.0	322 ± 5
BDME-10	14.4 ± 0.3	496 ± 4
Partizanka Niska	10.7 ± 0.2	361 ± 9
Triticum durum		
Kunduru-1149	10.6 ± 0.2	437 ± 15

In the Zn deficient plants, the total amounts of Zn varied only in a relatively narrow range (23.0-32.0 μg Zn [48 $plants]^{-1}$) and were not related to the Zn deficiency of the genotypes (*Table 6.5*). This was also true after considering the Zn reserves in the seeds. Accordingly, the deficient plants had taken up some Zn from the nutrient solution without Zn supply (impurities in water and added salts), but this uptake was not particularly related to the Zn efficiency of the genotypes.

Table 6.4. Zinc concentration in shoots and roots of 15 days old wheat genotypes grown with (+Zn= 1 mmol m^{-3}) and without (-Zn) Zn supply in nutrient solution

	Zn concentration (µg g^{-1} dry weight)			
	Shoots		Roots	
Genotype	+Zn	-Zn	+Zn	-Zn
Triticum aestivum				
Dagdas-94	60	4.3	92	8.3
Gerek-79	62	4.7	57	6.3
SBVD 1-21	54	5.0	65	6.7
SBVD 2-22	51	4.3	94	7.7
BDME-10	55	4.3	70	8.5
Partizanka Niska	51	5.0	71	7.9
Triticum durum				
Kunduru-1149	52	7.0	71	8.9
LSD (0.05)	5	0.5	6	0.9

The effect of Zn deficiency on release rate of phytosiderophores is shown in *Figure 6.1*. In all genotypes adequately supplied with Zn, release rate of phytosiderophores was very low throughout 15 days old growth and did not exceed 0.5 µmol (48 plants)$^{-1}$ 3 h^{-1} (data not shown). Also in Zn deficient durum wheat genotype Kunduru-1149, the release rate of phytosiderophore release was very low and remained at a maximum level of 1.2 µmol (*Figure 6.1*). However, in all Zn deficient bread wheat cultivars, the release of phytosiderophores was markedly increased, even in the Z inefficient bread wheat genotypes. The Zn deficiency-susceptible bread wheat cultivar SBVD 2-22 even showed the highest rate of phytosiderophore release (17.4 µmol) and the Zn inefficient cultivars BDME-10 and SBVD 1-21 released phytosiderophores nearly as high as the most Zn efficient cultivar Dagdas-94 (*Figure 6.1*). Among the bread wheat genotypes, Partizanka Niska (Zn inefficient), showed the lowest rate of phytosiderophore release under Zn deficiency. If the release rate of phytosiderophores is expressed per root dry weight, similar data are obtained for Zn efficient and Zn inefficient bread wheat genotypes, as their similar root dry weights (*Table 6.2*).

Table 6.5. Amounts of Zn in the Zn deficient wheat plants after 15 days growth without Zn supply (calculated from *Tables* 2, 3, and 4)

Genotype	With seed reserves	Without seed reserves
	μg Zn (48 plants)$^{-1}$	
Triticum aestivum		
Dagdas-94	32.0	9.5
Gerek-79	23.0	7.5
SBVD 1-21	30.2	4.6
SBVD 2-22	26.2	10.7
BDME-10	27.4	3.6
Partizanka Niska	24.6	7.3
Triticum durum		
Kunduru-1149	27.1	6.1

The root exudates released by Zn deficient plants were also analyzed by HPLC for identification of phytosiderophores and showed that 2′-deoxymugineic acid is the predominant phytosiderophore released from Zn deficient roots (see *Chapter 5*, for the diagrams of HPLC analysis of root exudates of bread wheats).

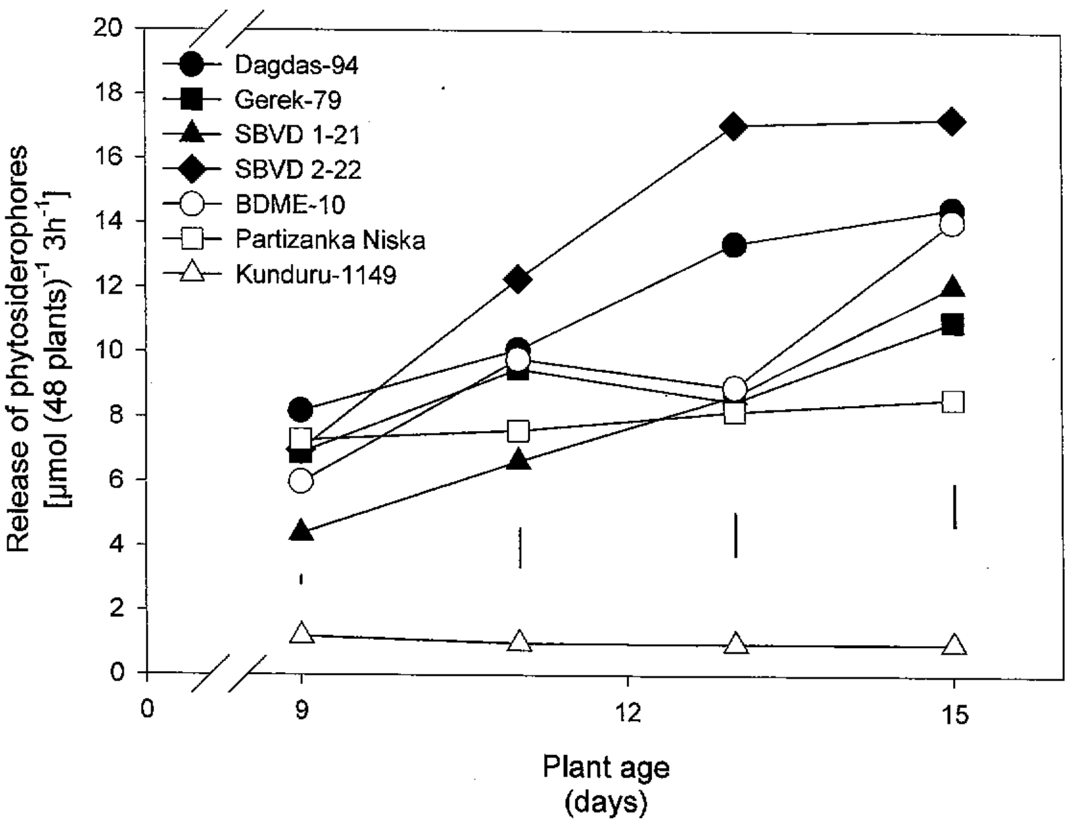

Figure 6.1. Effect of Zn deficiency on the rate of phytosiderophore release from roots of various wheat genotypes during 15 days growth in nutrient solution. Among the genotypes, Kunduru-1149 is a durum wheat and the others are bread wheat. Bars indicate LSD (0.05)

6.5. Discussion

The data presented in *Figure 6.1* on enhanced release of phytosiderophores from wheat roots in response to Zn deficiency confirm former results (Zhang et al., 1989; Cakmak et al., 1994; Walter et al., 1994). In Zn deficient bread wheat genotypes, the rates of phytosiderophore release were up to 35-fold higher than in Zn sufficient plants. In contrast, the release of phytosiderophores remained low in the durum wheat cultivar Kunduru-1149, which also had Zn deficiency. Durum wheat is particularly sensitive to Zn deficiency when grown in both soil and nutrient solution (Graham et al., 1992; Cakmak et al., 1996a, 1996b). Higher sensitivity of durum wheat that bread wheat to Zn deficiency has been attributed to their low capacity to synthesize and release phytosiderophores (Cakmak et al., 1994, 1996c) which is consistent with results presented here. Differences in the release rate of phytosiderophores were also considered as a suitable parameter for evaluation of Zn efficiency between cultivars of a given species such as bread wheat (Cakmak et al., 1996c). However, the results presented here show that the differential susceptibility of bread wheat cultivars to Zn deficiency does not correspond well with the capacity of the genotypes to release phytosideophores. For example, the most efficient (i.e., Dagdas-94) and Zn inefficient (i.e., BDME-10) bread wheat cultivars did not differ in their capacity to release phytosiderophores from roots. Similar to our results on Zn deficiency, the release of phytosiderophores is not always correlated with the sensitivity of different genotypes, as was noted for oat cultivars to Fe deficiency (Hansen and Jolley, 1995).

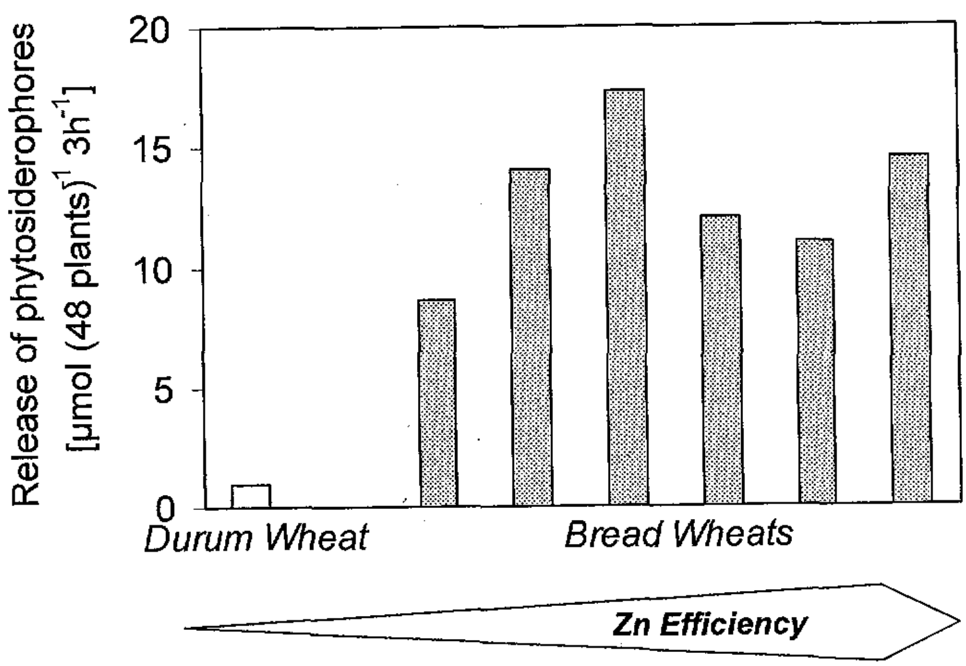

Figure 6.2. Relationship between phytosiderophore release and Zn efficiency of wheat genotypes grown for 15 days in nutrient solution without Zn supply

Apparently, additional mechanisms operate in the rhizosphere or within plants contributing to expression of Zn efficiency in cultivars of a species like bread wheat. In soil grown plants, the Zn deficiency-enhanced root growth of some Zn efficient cultivars (*Table 6.2*) might be a contributing factor to Zn efficiency as has also been suggested by Dong et al. (1995). Differences in uptake capacity of the roots and translocation of Zn to shoot meristems might be considered as an additional mechanism determining Zn efficiency in genotypes (Rengel and Graham, 1995b, 1996; Cakmak et al., 1996b). Higher Zn uptake capacity in Zn efficient genotypes compared to inefficient genotypes has been attributed to higher amounts of sulfhydryl groups in ion channels of plasma membranes has been suggested to be a critical step in Zn absorption into cells (Kochian, 1993; Welch, 1995). In maize genotypes, evidence has been presented that genotypes might have similar rates of phytosiderophore release, but markedly differ in their capacity to transport Zn-chelated phytosiderophores across the plasma membranes of root cells (von Wiren et al., 1996).

Alternatively, differences in internal utilization or mobility of Zn might be involved in expression of Zn efficiency. In many instances, susceptibility of genotypes to Zn deficiency greatly differs as was noted in this study, even though the genotypes have similar leaf or shoot Zn concentrations (Rengel and Graham, 1995b; Cakmak et al., 1996b). Differences in internal concentration of chelators for Zn such as certain amino acids (e.g., cysteine, methionine, and histidine) or nicotianamine might affect mobility of Zn in plants (Stephan et al., 1994; Welch, 1995), and perhaps also affect physiological availability of Zn at the tissue and cellular level. Recently, it has been shown that under Zn deficiency conditions and at the same total concentration of Zn in the leaves, activity of the Zn-containing enzyme carbonic anhydrase is much greater in Zn efficient than in Zn inefficient wheat cultivar, indicating higher levels of physiologically active Zn pool in leaves of Zn efficient genotypes (Rengel, 1995b).

In conclusion, the results presented in this study show that the release rate of phytosiderophores from roots of Zn deficient bread wheat genotypes does not always explain the differences in sensitivity of genotypes to Zn deficiency. Besides phytosiderophore release, differences in root uptake, and root-to-shoot transport of Zn and its distribution within shoots, and internal concentration of chelators affecting Zn mobility in plants and utilization at cellular level have to be considered in evaluation of wheat genotypes for Zn efficiency.

7. Uptake of zinc (^{65}Zn) from chelator-buffered nutrient solution by rye, bread wheat and durum wheat cultivars differing in zinc efficiency

7.1. Abstract

Effect of zinc (Zn) nutritional status on uptake of inorganic 65Zn was studied in rye (*Secale cereale*, cv. Aslim), three bread wheat (*Triticum aestivum*, cvs. Dagdas, Bezostaja, and BDME-10), and durum wheat (*Triticum durum*, cv. Kunduru-1149) cultivars grown for 13 days in nutrient solution under controlled environmental conditions. The cultivars were selected based on their response to Zn deficiency and to Zn fertilization in calcareous soils under field conditions. When grown in Zn-deficient calcareous soil in the field, the rye cultivar had the highest and the durum wheat had the lowest Zn efficiency. Among the bread wheat cultivars, BDME-10 showed higher susceptibility to Zn deficiency and Bezosaja and Dagdas were affected less by Zn deficiency. Similarly to field conditions, in nutrient solution visual Zn deficiency symptoms (i.e. necrotic lesions on leaf blade) appeared to be more severe in Kunduru-1149 and BDME-10 and less severe in rye cultivar Aslim. Under Zn deficiency, shoot concentrations of Zn were similar in all cultivars. Cultivars with adequate Zn supply did not differ in uptake and root-to-shoot translocation rate of ^{65}Zn, but under Zn deficiency there were distinct differences; rye showed the highest rate of Zn uptake and the durum wheat the lowest. In the case of bread wheat cultivars, ^{65}Zn uptake rate was about the same and not related to their differential Zn efficiency. Under Zn deficiency, rye had the highest rate of root-to-shoot translocation of ^{65}Zn, while all bread and durum wheat cultivars were similar in their capacity to

translocate ^{65}Zn from roots to shoots. When Zn^{2+} activity in uptake solution ranged between 117 p*M* and 34550 p*M*, Zn-efficient and Zn-inefficient bread wheat genotypes were again similar in uptake and root-to-shoot traslocation rate of ^{65}Zn.

The results indicate that high Zn efficiency of rye can be attributed to its greater Zn uptake capacity from soils. The inability of the durum wheat cultivar Kunduru-1149 to have a high Zn uptake capacity seems to be an important reason for its Zn inefficiency. Differential Zn efficiency between the bread wheat cultivars used in this study is not related to their capacity to take up inorganic Zn.

7.2. Introduction

Zinc (Zn) deficiency is a common nutritional problem for plants, particularly for cereals grown on calcareous soils of arid and semi arid regions, resulting in severe decreases in grain yield (Cakmak et al., 1996a; Graham et al., 1992).

In long-term experiments under field conditions Zn-efficient wheat genotypes had a higher Zn uptake capacity than Zn-inefficient genotypes (Cakmak et al., 1997a; Graham et al., 1992). In short-term experiments under controlled environmental conditions, Zn-efficient wheat genotypes also had a greater Zn uptake rate than Zn-inefficient genotypes (Cakmak et al., 1998; Rengel and Graham, 1996; Rengel and Wheal, 1997; Rengel et al., 1998). However, in most of these studies bread wheat genotypes having higher Zn efficiency were compared with durum wheat genotypes with lower Zn efficiency. The term "Zn efficiency" used in this paper reflects the ability of a cultivar to grow and yield well under Zn-deficient conditions.

Rengel and Wheal (1997) studied kinetic parameters of Zn uptake in bread wheat cultivars differing in Zn efficiency and showed that under Zn deficiency, Zn-efficient cultivar showed a greater Imax value (maximum net uptake rate) than Zn-inefficient cultivar. However, both cultivars were similar in their Km value. Recently, using the bread wheat cvs. Dagdas-94 and BDME-10 tested in this PhD thesis, Hacisalihoglu and his colleagues (2001) showed the presence of two separate Zn transport systems mediating high- and low-affinity Zn influx for both cultivars. However, they did not observe any difference between both genotypes neither in low-affinity nor in high-affinity activity ranges, showing a disaggrement with the results presented by Rengel and Wheal (1997). Recently, differences in Zn uptake capacity between bread and durum wheat cultivars were attributed to differential release of phytosiderophores from roots (Rengel et al., 1998). Phytosiderophores possess a high ability to complex Zn and enhance its mobility in the rhizosphere (Treeby et al., 1989) and root apoplast (Zhang et al., 1991). Phytosiderophores are also involved in uptake of Zn and its translocation to shoots (von Wiren et al., 1996). A greater rate of phytosiderophore release from roots of bread wheat over durum wheat was considered a major reason for higher Zn efficiency of bread wheat compared to durum wheat (Cakmak et al., 1994; Walter et al., 1994). However, differences in rate of phytosiderophore release were not always correlated well with differential Zn efficiency between and within cereal species (Cakmak et al., 1998; Erenoglu et al., 1996).

The aim of this study was to compare Zn uptake capacity of bread wheat cultivars differing in Zn efficiency under field conditions. A Zn-inefficient durum wheat and a highly Zn-efficient rye cultivar were also used for better evaluation of the relationship between Zn efficiency and Zn uptake capacity of cultivars. Zinc uptake experiments were carried out using labelled Zn (^{65}Zn) in nutrient solution.

7.3. Materials and methods

One rye (*Secale cereale* L. cv. Aslim), three bread wheat (*Triticum aestivum* L. cvs. Bezostaja, Dagdas, BDME-10) and one durum wheat (*Triticum durum* L. cv. Kunduru-1149) cultivars were used in experiments in nutrient solution 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 μmol m^{-2} s^{-1} at plant height provided by Sylvania FR 96 T lamps). The cultivars used in this study differ greatly in their Zn efficiency, and were selected based on their growth under field conditions in a severely Zn-deficient calcareous soil in Central Anatolia, Turkey. Among the bread wheat cultivars, BDME-10 has much higher sensitivity to Zn deficiency than Dagdas and Bezostaja, while the durum wheat and rye cultivars show the highest and lowest Zn efficiency among genotypes tested here (for more details see Cakmak et al., 1996a; 1997a).

Experiment I

Surface-sterilized seeds were germinated in quartz sand moistened with saturated $CaSO_4$ solution. After 5 d, seedlings were transferred to 2.5-L plastic pots (30 seedlings per pot) containing aerated nutrient solution prepared using the method described by Norvell and Welch (1993) and Rengel and Graham (1995). This solution contained (in mmol m^{-3}): 2000 $Ca(NO_3)_2$, 500 $MgSO_4$, 1500 KNO_3, 100 KCl, 2000 MES-KOH, 100 $NH_4H_2PO_4$, 10 H_3BO_3, 0.1 Na_2MoO_4, 25 K_3-(N-(2 hydroxyethyl) ethylenedinitrilotriacetic acid) (HEDTA), 100 FeHEDTA, 1 MnHEDTA, 0.5 CuHEDTA, and 0.1 NiHEDTA. Zinc was supplied as ZnHEDTA at concentrations of 0.1 μ*M* (Zn-deficient plants) and 10 μ*M* (Zn-sufficient plants). These total Zn concentrations corresponded to 2 and 200 p*M* Zn^{2+} activity, respectively.

On 9, 11, and 13 days, plants were transferred to 500 mL micronutrient free nutrient solution, and 8.0 mmol m^{-3} $ZnSO_4$ + 18.2 mmol m^{-3} HEDTA (Zn^{2+} activity= 386 p*M*), labelled with 37 KBq ^{65}Zn was added into this solution for 8h. Calculation of free Zn^{2+} activity was made by the GEOCHEM-PC program (Parker et al., 1995). The uptake experiment was started 6 h after the onset of the light period to avoid possible effects of phytosiderophores on Zn uptake, as the release of phytosiderophores from roots occurs between two and five hours after the onset of light period (Marschner et al., 1986). At the end of the uptake periods, roots were washed for 10 min using 250 mL of 1 m*M* $CaSO_4$ followed by 250 mL of 1 m*M* NaEDTA to remove extracellular Zn (von Wiren et al., 1996).

Experiment II

In another experiment bread wheat cultivars Dagdas and BDME-10 were used to study ^{65}Zn uptake capacity of these cultivars, when supplied with increasing activities of free Zn^{2+}. For this experiment, plants were grown under growth conditions described above without Zn application for 11 days and were then transferred into solution containing increasing free activities of Zn^{2+} in 500 mL chelator-buffered nutrient solution for 24 h. Concentrations of $ZnSO_4$ and HEDTA activities of free Zn^{2+} and the ^{65}Zn radioactivities are given in *Table 7.1.*

Table 7.1. Concentration of $ZnSO_4$, Zn^{+2} activity and ^{65}Zn radioactivity in uptake solution. All solutions contained 18.2 mmol m^{-3} HEDTA. Free Zn^{2+} activity was calculated by GEOCHEM-PC program (Parker et al., 1995)

$ZnSO_4$ concentration (mmol m^{-3})	Zn^{2+} activity (p*M*)	^{65}Zn radioactivity (KBq)
3.5	117	154
8.0	386	154
16.0	3586	61
18.0	34550	31

Following termination of experiments, roots and shoots were harvested, dried and ashed at 550 °C. The ashed samples were dissolved in HCl and assayed for Zn concentration by atomic absorption spectroscopy and for ^{65}Zn by liquid scintillation spectrometry.

7.4. Results

Appearance of leaf symptoms of Zn deficiency, such as necrotic patches on leaf blades, started after 9 days of growth in nutrient solution with low (Zn^{2+} activity=2 p*M*) Zn supply and became more visible on day 13. Similar to field observations on Zn-deficient calcareous soils, severe leaf symptoms of Zn deficiency appeared first in Kunduru and BDME-10. The rye cultivar was the least affected by Zn deficiency, while the bread wheat cultivars Bezostaja and Dagdas showed a moderate sensitivity as judged from the severity of visual symptoms of Zn deficiency.

Appearance of leaf symptoms on day 9 was associated with slight decreases in shoot and root dry matter production (*Table* 7.2). Decreases in dry matter production due to Zn deficiency became more distinct with time, especially for shoot growth of Kunduru and BDME-10. In all cultivars, root growth was less affected by Zn deficiency than shoot growth (*Table* 7.2). As expected, shoot and root concentrations of Zn were higher in plants with adequate than deficient supply of Zn (*Table* 7.3). Under Zn-deficient conditions, cultivars did not differ clearly in shoot and root Zn concentrations (*Table* 7.3).

Compared to plants with adequate Zn supply, Zn-deficient plants had a much higher ^{65}Zn uptake rate (*Figure 7.1*). At adequate supply of Zn, all cultivars were similar in their capacity to take up Zn. However, under Zn-deficient conditions the rate of Zn uptake markedly differed between the cultivars. Zinc-deficient rye and durum wheat cultivars showed the highest and the lowest ^{65}Zn uptake rate, respectively, while bread wheat cultivars were intermediate in the ^{65}Zn uptake rate. No clear difference could be found in the ^{65}Zn uptake rate between bread wheat cultivars. Effects of Zn deficiency on the rate of root-to-shoot translocation of ^{65}Zn (*Figure* 7.2) were similar to effects on ^{65}Zn uptake rate by roots (*Figure* 7.1). Rye showed a greater rate of ^{65}Zn translocation than all wheat cultivars, but there were little differences in ^{65}Zn translocation rate between wheat cultivars (*Figure* 7.2).

Table 7.2. Shoot and root dry weight of rye, bread wheat, and durum wheat cultivars grown for 9, 11, and 13 days with deficient (Zn^{2+} activity = 2 p*M*) and sufficient (Zn^{2+} activity = 200 p*M*) Zn supply in nutrient solution. The data represent means ± SD of three independent replications

	Day 9		Day 11		Day 13	
Species/cultivars	Deficient	Sufficient	Deficient	Sufficient	Deficient	Sufficient
			[mg (plant part)$^{-1}$]			
			SHOOT			
Secale cereale						
Aslim	27 ± 2	32 ± 1	35 ± 1	46 ± 3	44 ± 1	56 ± 2
Triticum aestivum						
Bezostaja	25 ± 1	31 ± 3	29 ± 6	47 ± 3	41 ± 3	57 ± 5
Dagdas-94	20 ± 3	26 ± 2	26 ± 2	38 ± 1	36 ± 3	46 ± 7
BDME-10	19 ± 3	28 ± 1	23 ± 1	38 ± 3	28 ± 3	46 ± 7
Triticum durum						
Kunduru-1149	28 ± 4	39 ± 2	32 ± 3	51 ± 2	39 ± 4	74 ± 4
			ROOT			
Secale cereale						
Aslim	9 ± 1	8 ± 1	11 ± 2	13 ± 2	13 ± 2	10 ± 1
Triticum aestivum						
Bezostaja	12 ± 1	10 ± 1	14 ± 2	16 ± 2	18 ± 2	15 ± 2
Dagdas-94	11 ± 2	9 ± 1	16 ± 1	11 ± 1	18 ± 2	15 ± 2
BDME-10	12 ± 1	9 ± 1	13 ± 1	11 ± 1	15 ± 1	12 ± 2
Triticum durum						
Kunduru-1149	14 ± 1	12 ± 1	18 ± 1	17 ± 2	24 ± 1	21 ± 2

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Table 7.3. Concentration of Zn in shoot and root of rye, bread wheat, and durum wheat cultivars grown for 9, 11, and 13 days with deficient (Zn^{2+} activity = 2 p*M*) and sufficient (Zn^{2+} activity = 200 p*M*) Zn supply in nutrient solution. The data represent means ± SD of three independent replications

	Day 9		Day 11		Day 13	
Species/cultivars	Deficient	Sufficient	Deficient	Sufficient	Deficient	Sufficient
			[mg (*plant part*)$^{-1}$]			
			SHOOT			
Secale cereale						
Aslim	11 ± 1	55 ± 4	10 ± 1	50 ± 3	9 ± 1	48 ± 2
Triticum aestivum						
Bezostaja	11 ± 1	49 ± 1	8 ± 1	48 ± 2	8 ± 1	46 ± 3
Dagdas-94	10 ± 1	53 ± 5	8 ± 1	53 ± 4	8 ± 1	52 ± 4
BDME-10	10 ± 1	59 ± 3	8 ± 1	58 ± 5	8 ± 1	57 ± 6
Triticum durum						
Kunduru-1149	10 ± 1	56 ± 6	8 ± 1	57 ± 3	8 ± 1	58 ± 8
			ROOT			
Secale cereale						
Aslim	20 ± 2	62 ± 4	15 ± 2	65 ± 7	16 ± 1	60 ± 3
Triticum aestivum						
Bezostaja	22 ± 1	53 ± 2	20 ± 3	56 ± 5	20 ± 3	57 ± 6
Dagdas-94	18 ± 2	54 ± 5	18 ± 4	58 ± 3	19 ± 4	59 ± 5
BDME-10	21 ± 4	62 ± 7	19 ± 1	60 ± 4	20 ± 2	59 ± 4
Triticum durum						
Kunduru-1149	16 ± 1	48 ± 9	18 ± 3	52 ± 5	18 ± 2	53 ± 7

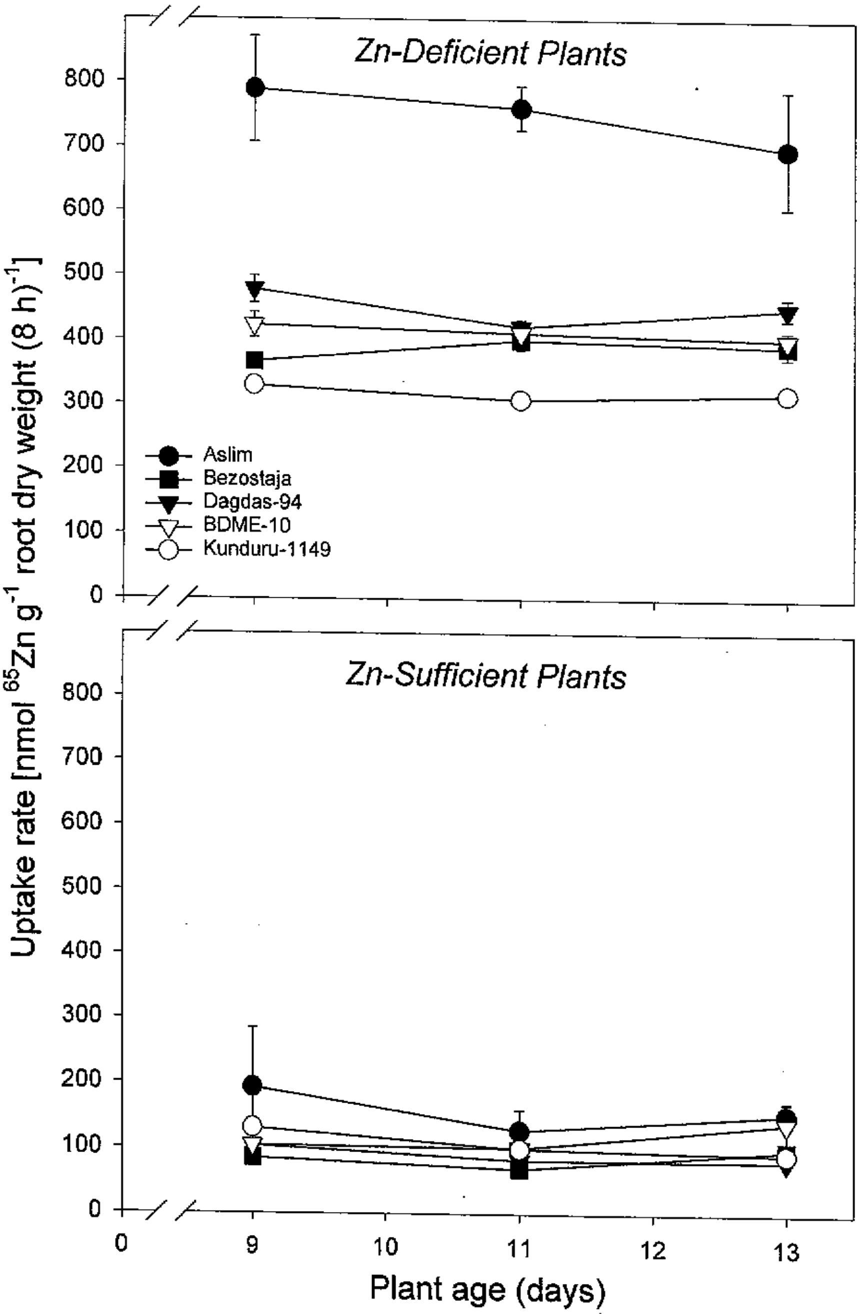

Figure 7.1. Uptake rates of ^{65}Zn in 9, 11, and 13 day-old rye (cv. Aslim), bread wheat (cvs. Bezostaja, Dagdas-94, BDME-10) and durum wheat (cv. Kunduru-1149) over 8 hours. Plants were grown in nutrient solution with sufficient and deficient Zn supply. Data represent means ± SD of three independent replicates

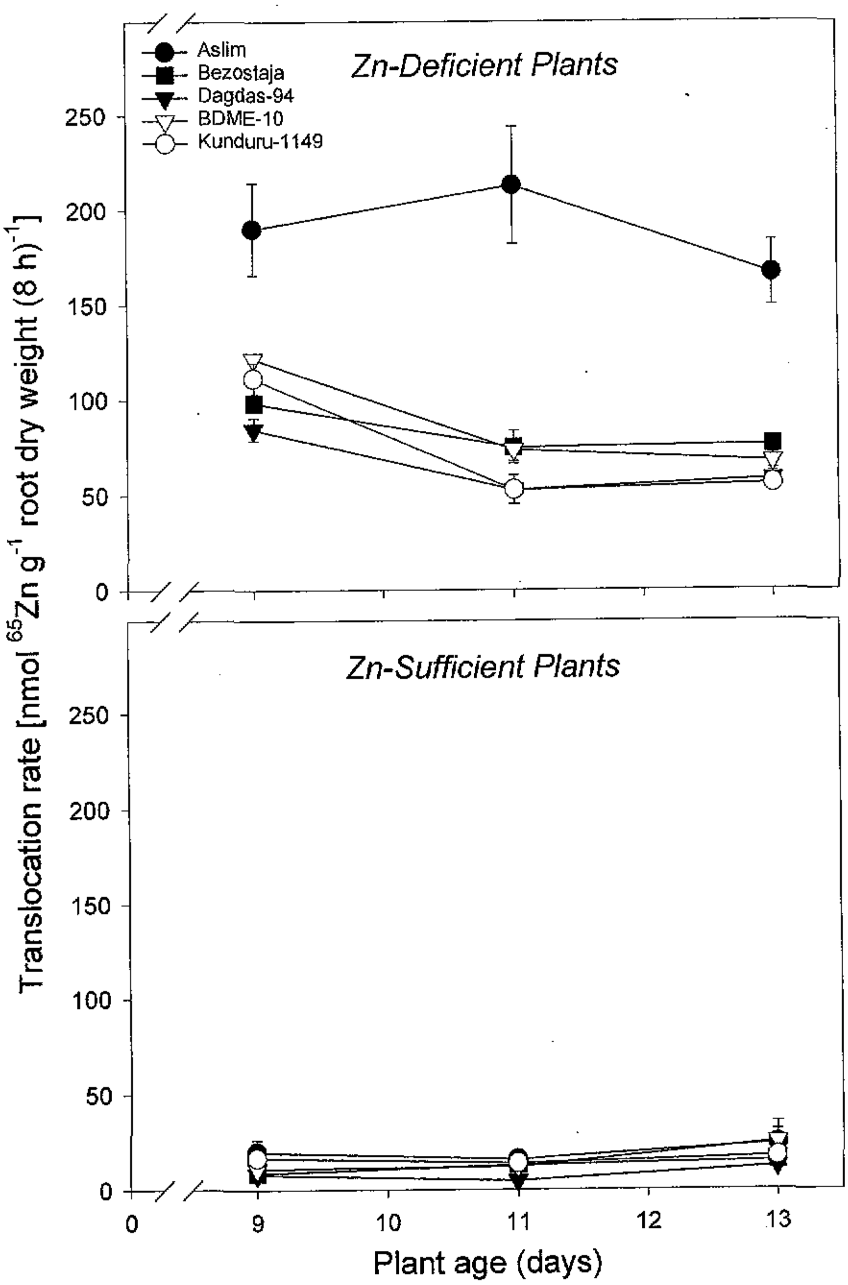

Figure 7.2. Root-to-shoot translocation rates of ^{65}Zn in 9, 11, and 13 day-old rye (cv. Aslim), bread wheat (cvs. Bezostaja, Dagdas-94, BDME-10) and durum wheat (cv. Kunduru-1149) over 8 hours. Plants were grown in nutrient solution with sufficient and deficient Zn supply. Data represent means±SD of three independent replicates

Of the bread wheats, Dagdas (higher Zn efficiency) was compared with BDME-10 (lower Zn efficiency) for their capacity to take up Zn from nutrient solution containing varied Zn activities. Irrespective of Zn^{2+} activity in nutrient solution, Dagdas and BDME-10 did not differ in rates of ^{65}Zn uptake and translocation to shoots (*Figure 7.3*).

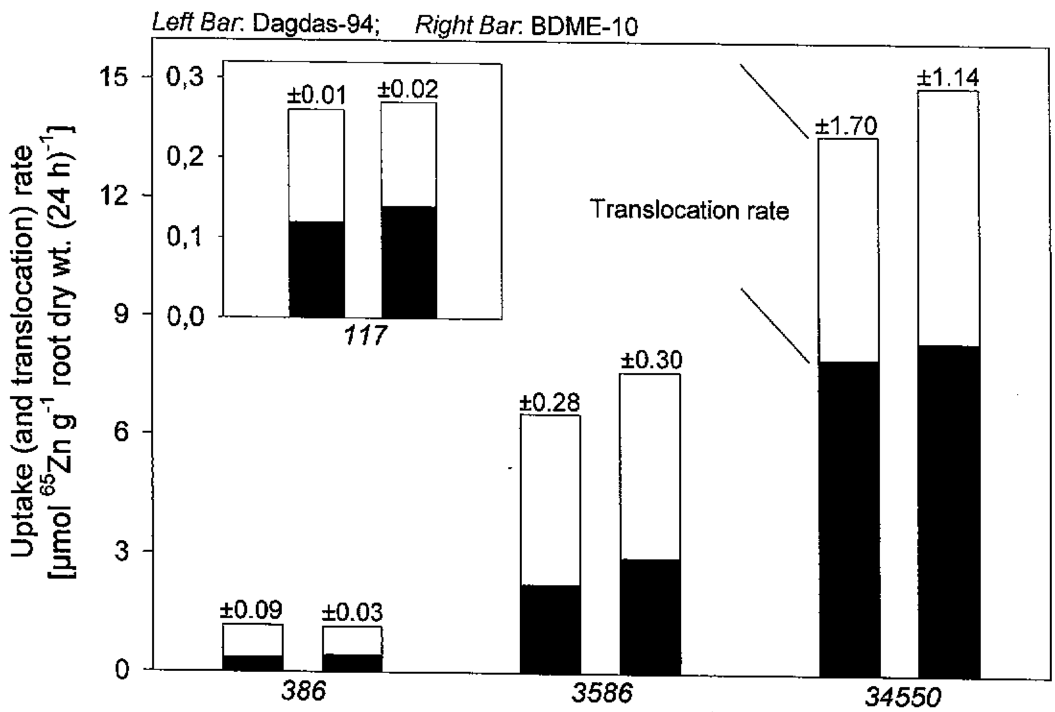

Figure 7.3. Effect of increasing supply of Zn^{2+} activity labelled with ^{65}Zn on uptake rate of Zn by roots and its translocation to shoots in the bread wheat cultivars Dagdas-94 and BDME-10 grown for 11 days in nutrient solution. The data represent means of three independent replicates. ±SD values of uptake rates are given on the top of bars

7.5. Discussion

Among the cultivated cereal species, rye is known to have an exceptionally high Zn efficiency (Cakmak et al., 1997a; Ekiz et al., 1998). The results presented in *Figure 7.1* suggest that enhancement in the Zn uptake rate under Zn-deficient conditions is the important plant mechanism determining expression of high Zn efficiency in rye. Higher Zn uptake capacity of rye than of other cereals (*Figure 7.1*) was also found in long-term experiments in the field by calculating Zn accumulation per plant (Cakmak et al., 1997a; Ekiz et al., 1998). Despite its greater capacity for uptake and shoot translocation of Zn, rye grown under Zn deficiency in the field is unable to achieve high concentration in leaf or shoot tissue (Cakmak et al., 1997a), probably because increases in Zn uptake cause an additional dry matter production with corresponding dilution of Zn present in tissue (Marschner, 1995).

In contrast to rye, durum wheat is the most sensitive cereal species to Zn deficiency (Cakmak et al., 1997a; Graham and Rengel, 1993; Rengel and Graham, 1995). In full agreement with the findings of Rengel and Wheal (1997) and Rengel et al. (1998), high sensitivity of durum wheats to Zn deficiency is possibly attributable to their lower capacity to take up Zn under Zn-deficient conditions (Figure 1). Poor ability of durum wheats to accumulate Zn was also shown in long-term experiments under field conditions (Cakmak et al., 1997a; Graham et al., 1992).

The reason for a higher Zn uptake rate of rye under deficient supply of Zn is not understood at present. According to Rengel and Hawkesford (1997) and Rengel and Wheal (1997), expression of higher Zn uptake capacity in Zn-efficient bread wheat than Zn-inefficient durum wheat genotype is closely associated with expression of a 34-kDa polypeptide in the root-cell plasma membranes. This polypeptide may potentially be the main structural unit of a putative plasma membrane transporter for Zn. Recently, it has been proposed that uptake of free Zn cation in maize plants is controlled by a putative Zn-transporter protein (von Wiren et al., 1996). It is therefore of high physiological importance to study whether a similar polypeptide or a transporter protein mediating Zn influx is expressed in root-cell plasma membranes of rye plants under Zn-deficient conditions. Induction of a Zn-transporter protein by Zn deficiency is known for yeast cells (Zhao and Eide, 1996), and could be a major plant trait for triggering increased Zn efficiency in higher plants.

Differences in the Zn uptake rate are also known within genotypes of a given cereal species such as rice (Bowen, 1986) and sorghum (Ramani and Kannan, 1985). However, the results obtained in these studies have little relevance to Zn efficiency of rice and sorghum genotypes, because Zn uptake experiments were carried out with plants that had been supplied unusually high Zn concentrations (up to 0.5 mol m^{-3}). By contrast, using physiologically relevant concentrations of Zn in nutrient solution (0 to 2 μM), Rengel and Wheal (1997) recently compared two bread wheat cultivars for their Zn uptake kinetics. Zinc-efficient bread wheat cultivar had only a 30% higher rate of net Zn uptake than Zn-inefficient bread wheat. In the present study, 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 ^{65}Zn (*Figs. 7.1 and 7.2*). Such minor inconsistency might have resulted from different experimental conditions and different bread wheat cultivars used by Rengel and Wheal (1997) and in the present study. The results of this study lead us to conclude that the bread wheat cultivars differing in Zn efficiency did not differ in their capacity to take up Zn when grown under Zn-deficient conditions. Interestingly, the release rate of PS's also could not explain well the genotypic variation in Zn efficiency between bread wheat cultivars (Erenoglu et al., 1996). However, when compared with durum wheats, bread wheats showed a higher rate of Zn uptake and PS release (Cakmak et al., 1996b; Rengel et al., 1998).

Obviously, additional mechanisms are involved in expression of high Zn efficiency within bread wheat cultivars. Differences in internal utilization of Zn would be one plausible alternative explanation for differential expression of Zn efficiency within bread wheat cultivars. Based on the measurement of activity of Zn-containing superoxide dismutase (SOD), it has been suggested that Zn-efficient bread wheat cultivars contain higher amounts of physiologically active Zn than Zn-inefficient bread wheat cultivars (Cakmak et al., 1997b, 1998). However, the magnitude of the difference in Zn-containing SOD activity between bread wheat cultivars was not high enough to explain genotypic differences in Zn efficiency. Further studies are needed for to elucidate the reasons for the differential expression of Zn efficiency among bread wheats. Moreover, maintaining sulfhydryl groups in root-cell plasma membranes in non-oxidized state was suggested to be one possible mechanism for higher Zn uptake capacity of Zn-efficient genotypes (Rengel, 1995; Rengel and Wheal, 1997). Existence of sulfhydryl groups in the reduced form is assumed to be a prerequisite for the maintenance of Zn uptake across the plasma membranes (Kochian, 1993; Welch, 1995). The level of non-oxidized sulfhydryl groups is decreased by Zn deficiency (Welch and Norvell, 1993), more clearly in roots of Zn-inefficient than Zn-efficient genotypes (Rengel, 1995), possibly due to oxidation of sulphydryl groups into disulphides by oxygen free radicals (Cakmak and Marschner, 1988).

From the results presented in this chapter, it can be concluded that higher Zn efficiency of rye in comparison to wheat species is closely related to its greater Zn uptake capacity under Zn-deficient conditions. Similarly, differences in Zn efficiency between bread and durum wheats can be ascribed to higher capacity of bread wheat to take up Zn when compared to durum wheat. However, variation in Zn efficiency between bread wheat cultivars can not be explained fully by differences in Zn uptake rates of cultivars, indicating existence of further mechanisms underlying high Zn efficiency in the bread wheat cultivars used in this study.

8. Uptake and transport of foliar applied zinc (^{65}Zn) in bread and durum wheat cultivars differing in zinc efficiency

8.1. Summary

Using two bread wheat (*Triticum aestivum*) and two durum wheat (*Triticum durum*) cultivars differing in zinc (Zn) efficiency, uptake and translocation of foliar-applied ^{65}Zn were studied to characterize the role of Zn nutritional status of plants on the extent of phloem mobility of Zn and to determine the relationship between phloem mobility of Zn and Zn efficiency of the used wheat cultivars. Irrespective of leaf age and Zn nutritional status of plants all cultivars showed similar Zn uptake rates with application of $^{65}ZnSO_4$ to leaf strips in a short term experiment. Also with supply of $^{65}ZnSO_4$ by immersing the tip (3 cm) of the oldest leaf of intact plants no differences in Zn uptake could be observed among and within both wheat species. Further, Zn nutritional status did not affect total uptake of foliar applied Zn. However, Zn-deficient plants translocated more ^{65}Zn from the treated leaf to the roots and remainder parts of shoots. In Zn-deficient plants about 40 % of the total absorbed ^{65}Zn was translocated from the treated leaf to the roots and remainder parts of shoots within 8 days, while in Zn-sufficient plants the proportion of the translocated ^{65}Zn of the total absorbed ^{65}Zn was about 25 %. Although differences in Zn efficiency existed between the cultivars did not affect the translocation and distribution of ^{65}Zn between roots and shoots. Bread wheats compared to durum wheats, tended to accumulate more ^{65}Zn in shoots and less ^{65}Zn in roots, particularly under Zn-deficient conditions.

The results indicate that differences in expression of Zn efficiency between and within durum and bread wheats are not related to translocation or distribution of foliar-applied ^{65}Zn within plants. Differential compartementation of Zn at the cellular levels is discussed as a possible factor determining genotypic variation in Zn efficiency within wheat.

8.2. Introduction

Zinc deficiency is one of the most common micronutrient deficiency in cereals, especially in wheat, significantly limiting grain yield, for example in Australia (Graham et al., 1992), India (Takkar and Walker, 1993), and Turkey (Cakmak et al., 1996a; 1999a). According to White and Zasoski (1999) the extent of Zn deficiency in soils is comparable with the extent of many macronutrient deficiencies causing large economic losses.

There are substantial differences in susceptibility to Zn deficiency between cereal species and also among cultivars of a given species. The susceptibility of cereals to Zn deficiency was found to decline in the order: durum wheat > oat ≥ bread wheat > barley > triticale > rye (Cakmak et al., 1997; Ekiz et al., 1998). Also diploid (AA), tetraploid (BBAA) and hexaploid (BBAADD) wheats differ greatly in their Zn efficiency (i.e., better growth and yield under Zn-deficient conditions). Among the diploid, tetraploid, and hexaploid wheats, the susceptibility of species to Zn deficiency declines in the order: tetraploid > hexaploid > diploid. Of the wheat genomes, A and B genomes exert increasing and decreasing effects on expression of high Zn efficiency, respectively (Cakmak et al., 1999b,c). Wheat is a peculiar cereal species showing great genotypical differences in susceptibility to Zn deficiency (Graham et al., 1992; Cakmak et al., 1996a; Torun et al., 2000).

The physiological basis of differential Zn efficiency among and within bread and durum wheats is not well characterized. Several mechanisms have been investigated to understand the physiological basis of differential Zn efficiency of wheat genotypes, including differences in root morphology (Dong et al., 1995), release of Zn-mobilising root exudates (phytosiderophores) (Cakmak et al., 1996c; Erenoglu et al., 1996), root uptake and root-to-shoot translocation of Zn (Rengel and Graham, 1996; Cakmak et al., 1998; Erenoglu et al., 1999), physiological availability of Zn at the cellular level (Rengel, 1995; Cakmak et al., 1997; Cakmak, 2000) and retranslocation of Zn (Erenoglu et al., 2001). In most cases, differences in sensitivity to Zn deficiency within and among bread and durum wheats were not related well to the uptake rate of Zn by roots (Rengel and Wheal, 1997; Cakmak et al., 1998; Erenoglu et al., 1999) and Zn concentrations of leaves or whole shoots (Graham et al., 1992; Rengel, 1995; Cakmak et al., 1996b, 1997; Torun et al., 2000). Based on these results it has been suggested that Zn-efficient bread wheat and Zn-inefficient durum wheat cultivars may differ in internal utilization and mobility of Zn, because of higher internal amounts of phytosiderophores in Zn-efficient bread cultivars (Cakmak et al., 1996c). Recently, Torun et al. (2000) assumed that in Zn-efficient bread wheat cultivars, Zn could possibly be retranslocated in greater amounts from older leaves into meristematic tissues allowing better and continuous growth under inadequate supply of Zn. The role of an efficient nutrient remobilization and re-translocation from older leaves into younger tissues in expression of a high nutrient use efficiency was shown for Ca-efficient cowpea genotypes (Horst et al., 1992).

The objectives of this paper were (i) to compare bread and durum wheat cultivars differing in Zn efficiency for their ^{65}Zn uptake capacity by leaves, (ii) to follow long-distance transport of ^{65}Zn applied to the tip of the oldest leaf, and (iii) to characterize the relationships between Zn efficiency of the cultivars, translocation of ^{65}Zn, and distribution of the translocated ^{65}Zn between roots and shoots under inadequate and sufficient supply of Zn.

8.3. Materials and Methods

Two bread wheat (*Triticum aestivum* L., cvs. Bezostaja and BDME-10) and two durum wheat (*Triticum durum* L., cvs. Kunduru-1149 and C-1252) cultivars were used in the experiments. Among these cultivars Bezostaja has been classified as Zn-efficient and other cultivars as Zn-inefficient when grown in calcareous soils low in available Zn (Kalaycı et al., 1999). Plants were grown in a nutrient solution under controlled climatic conditions (light/dark regimes of 16/8 h, temperature 24/20 °C and a photosynthetic photon flux density of 350 µmol m^{-2} s^{-1} at plant height). After germination in quartz sand wetted with $CaSO_4$ solution, seedlings were transferred to 2.5-L plastic vessels containing the following continuously aerated nutrient solution (in mol m^{-3}): 0.88 K_2SO_4, 2.0 $Ca(NO_3)_2$, 0.25 KH_2PO_4, 1.0 $MgSO_4$, 0.1 KCl, and as mmol m^{-3} 100 FeEDTA, 1 H_3BO_3, 0.5 $MnSO_4$, 0.2 $CuSO_4$, and 0.02 $(NH_4)_6Mo_7O_{24}$. Zinc was supplied in form of $ZnSO_4$ at concentrations of 0 mmol m^{-3} (for Zn-deficient plants) and 1 mmol m^{-3} (for Zn-sufficient plants). In the experiments dealing with Zn uptake by leaf strips, Zn-deficient plants were precultured with 0.01 µ*M* during the first 3 days in nutrient solution, and then without Zn supply to avoid severe deficiency symptoms in the oldest leaf.

Uptake of ^{65}Zn by leaf strips

After 8 days of growth in nutrient solution with different Zn supplies, the leaf strips from young and old leaves were prepared in a size 3x10 mm, separately, and washed in a solution containing 10 mol m^{-3} MES (pH 6.0) and 0.5 mol m^{-3} $CaSO_4$. One part of the leaf strip was infiltrated in the above solution under vacuum at -25 MPa (twice for 5 minutes), and the another part of the leaf strip was treated with the same solution but in the presence of 0.03 % L-77, a leaf surface wetting agent, without vacuum for 3 min. Thereafter, the uptake experiment was initiated by incubating about 100 mg (FW) leaf strips in 2 ml of uptake solution containing 10 mol m^{-3} MES (pH

6.0), 0.5 mol m^{-3} $CaSO_4$ and 0.01 mol m^{-3} $^{65}ZnSO_4$ (1415 kBq ^{65}Zn L^{-1}) with continuous shaking (125 rpm) for 60 min. After the incubation period, leaf strips were washed for 1 min in a solution containing 10 mol m^{-3} MES (pH 6.0) and 1 mol m^{-3} $CaSO_4$. Using the same wash solution, leaf samples were shaken on a reciprocal shaker for 10 minutes to remove apoplasmic ^{65}Zn. Finally, the samples were rinsed with 0.5 mol m^{-3} $CaSO_4$ for 2 minutes to remove ^{65}Zn present on the surface of leaf strips. Leaf strips were then dried, ashed and dissolved in 1 % HCl and analysed for ^{65}Zn by a liquid scintillator counter.

Some samples of precultured plants were collected to measure Zn concentrations in the oldest leaf (first leaf), young expanding leaf (second leaf), remainder of shoot and roots. Measurement of Zn was done after ashing the dried and ground samples at 500 °C by atomic absorption spectrometry.

Uptake and translocation of foliar-applied ^{65}Zn

After 6 days growth in nutrient solution with varied Zn supplies, 3 cm tip sections of the oldest leaf tips of intact plants were immersed in ^{65}Zn solution (1750 µl) in a 2ml Eppendorf tube for 10 seconds. This foliar application was undertaken 6 h before the beginning of the night period in the growth chamber. This application procedure was replicated two additional times in the next day during the light period. The application solution contained 0.1 mol m^{-3} $^{65}ZnSO_4$ (111 KBq ^{65}Zn in 1750 µL containing 0.01 % L-77 pH 6.0). Four and eight days after ^{65}Zn application, plants were harvested. The ^{65}Zn-treated leaf tips were cut and washed for about 10 min in 10 mol m^{-3} $ZnSO_4$ solution to remove ^{65}Zn adsorbed on the leaf surface and within the leaf apoplasmic spaces. The plants were divided in 4 parts: i) applied part (tip) of the treated oldest leaf, ii) non-applied part of the treated oldest leaf, iii) remainder of shoot, and iv) roots. As no ^{65}Zn could be detected in the non-applied part of the treated oldest leaf, these samples were not used in evaluation of the results. Results are presented as percentage of the total uptake of ^{65}Zn present in the treated leaf tip, remainder of shoot and roots.

8.4. Results

Zinc supply during preculture markedly enhanced concentration of Zn in shoot and root dry matter of all cultivars (*Table 8.1*). Both under deficient and sufficient supply of Zn the oldest leaves (first leaves) contained highest concentration of Zn when compared to remainder of shoots. At deficient supply of Zn, concentrations of Zn in remainder of shoots of all cultivars ranged between 10 to 16 mg kg^{-1} dry weight which are below the critical deficiency level (Marschner, 1995). Plants under Zn deficiency did not develop visual Zn deficiency symptoms and shoot growth was not affected (data not shown). However, root growth of all wheat cultivars tended to increase at nil Zn supply (9-21%) causing a lower shoot/root dry weight ratio in Zn-deficient plants. Lower shoot to root dry weight ratio is a well-known response of wheats to Zn deficiency (Cakmak et al., 1996b; Rengel and Graham, 1995a)

Similar uptake rates were obtained by using both techniques for ^{65}Zn application. This indicates that use of surface wetting agent, L-77, had no effect on ^{65}Zn uptake indicating that L-77 was not toxic for wheat plants at the concentration used of 0.03 % (*Figure 8.1*). Durum and bread wheats did not differ in uptake of ^{65}Zn by leaf strips (*Figure 8.1*). Also bread wheats cultivars differing in Zn efficiency were very similar in their ^{65}Zn uptake capacity via leaf strips. Further leaf age and Zn nutritional status did not affect uptake of ^{65}Zn (*Figure 8.1*). Similar to leaf strips, Zn nutritional status of plants did not affect the total uptake of ^{65}Zn in oldest leaves of intact plants (*Figure 8.2*).

Table 8.1. Effect of Zn supply on the concentration of Zn in different plant parts of the bread and durum wheat seedlings grown in nutrient solution for 11 days. Data represent means±SE of three independent replicates

Genotypes	Zn concentration (mg Zn kg^{-1} dry wt.)							
	First leaf		Second Leaf		Remainder of shoot		Roots	
	+Zn	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn	-Zn
Triticum aestivum								
Bezostaja	64 ± 6	18 ± 1	48 ± 3	12 ± 1	54 ± 3	16 ± 1	62 ± 9	12 ± 1
BDME-10	99 ± 7	20 ± 2	58 ± 2	14 ± 1	72 ± 4	16 ± 0	105 ± 7	14 ± 1
Triticum durum								
Kunduru-1149	109 ± 9	17 ± 3	62 ± 12	12 ± 1	61 ± 2	10 ± 1	57 ± 4	11 ± 0

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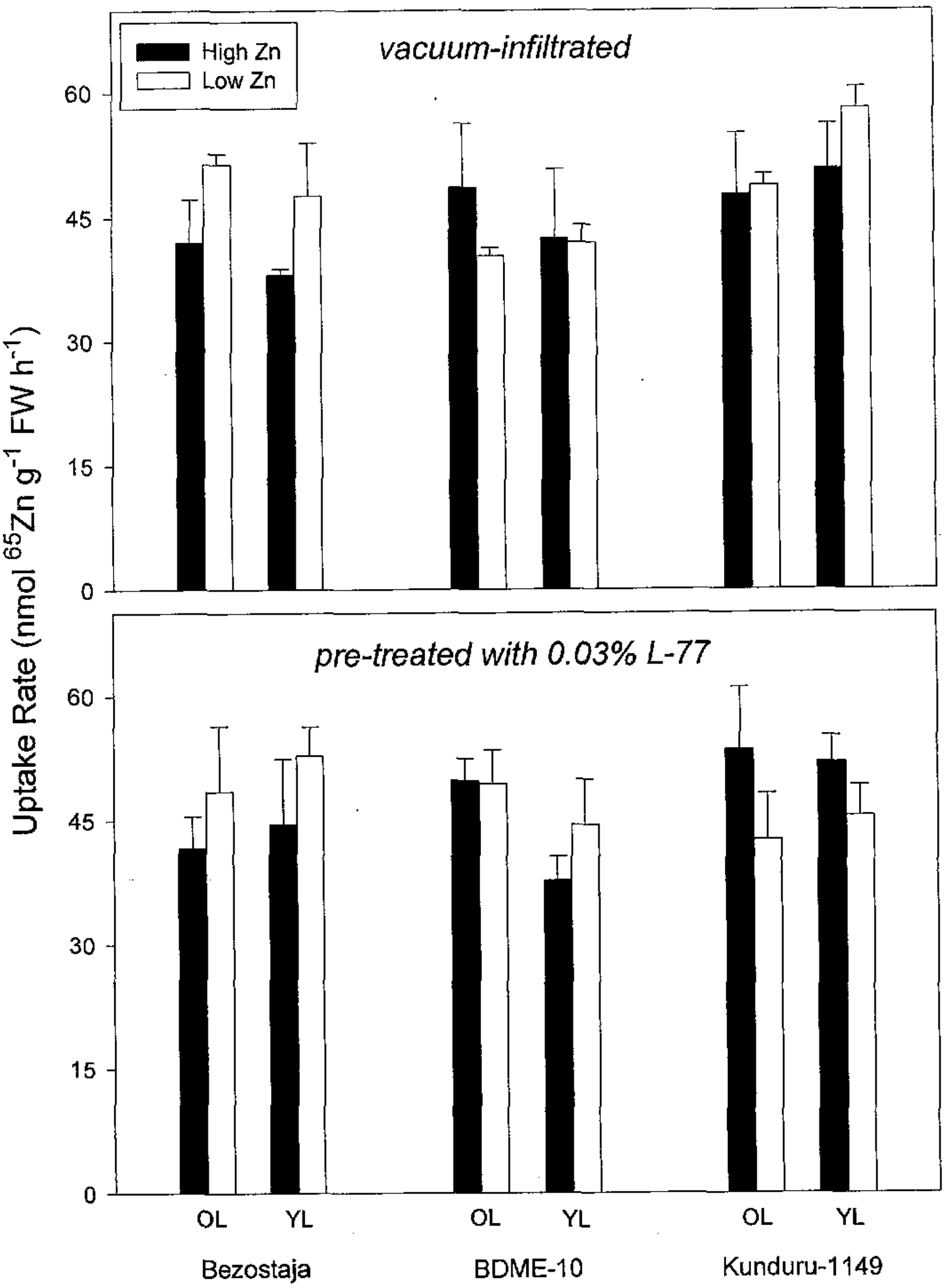

Figure 8.1. Uptake rate of ^{65}Zn by leaf strips of bread wheat (Bezostaja and BDME-10) and durum wheat (Kunduru-1149) cultivars differing in Zn efficiency grown for 8 days in nutrient solutionn supply (+/- Zn). Leaf strips were pretreated with a MES buffered $CaSO_4$ solution either by vacuum infiltrate (twice for five minutes) or by amendment with wetting-agent L-77. Data represent means±SD of four independent replicates (OL: old leaves, YL: young leaves)

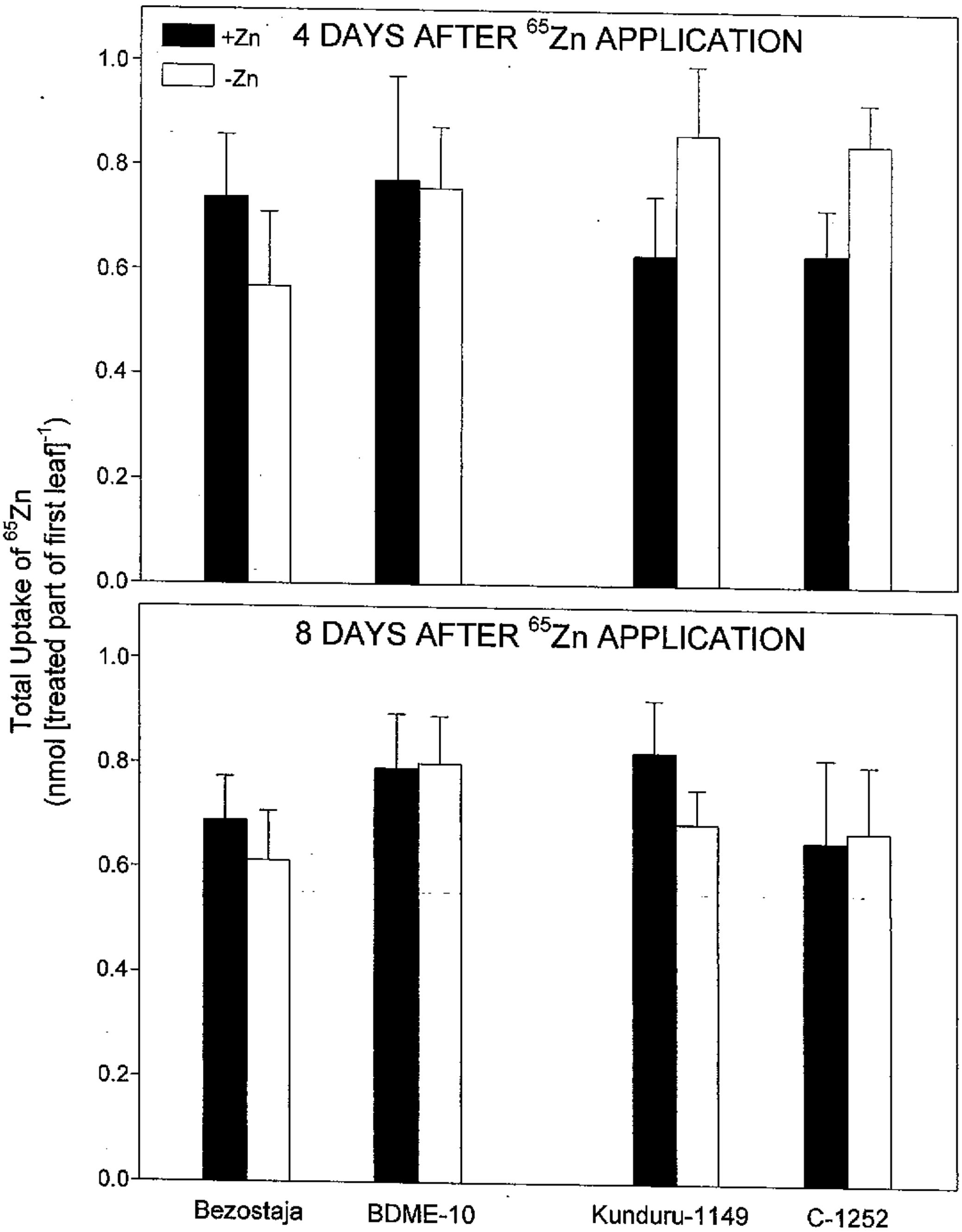

Figure 8.2. Total uptake of ^{65}Zn 4 and 8 days after application of ^{65}Zn to the upper part (3 cm) of the first leaf of bread wheat (Bezostaja and BDME-10) and durum wheat (Kunduru-1149 and C-1252) genotypes differing in Zn efficiency grown in nutrient solution. Data represent means±SD of four independent replicates

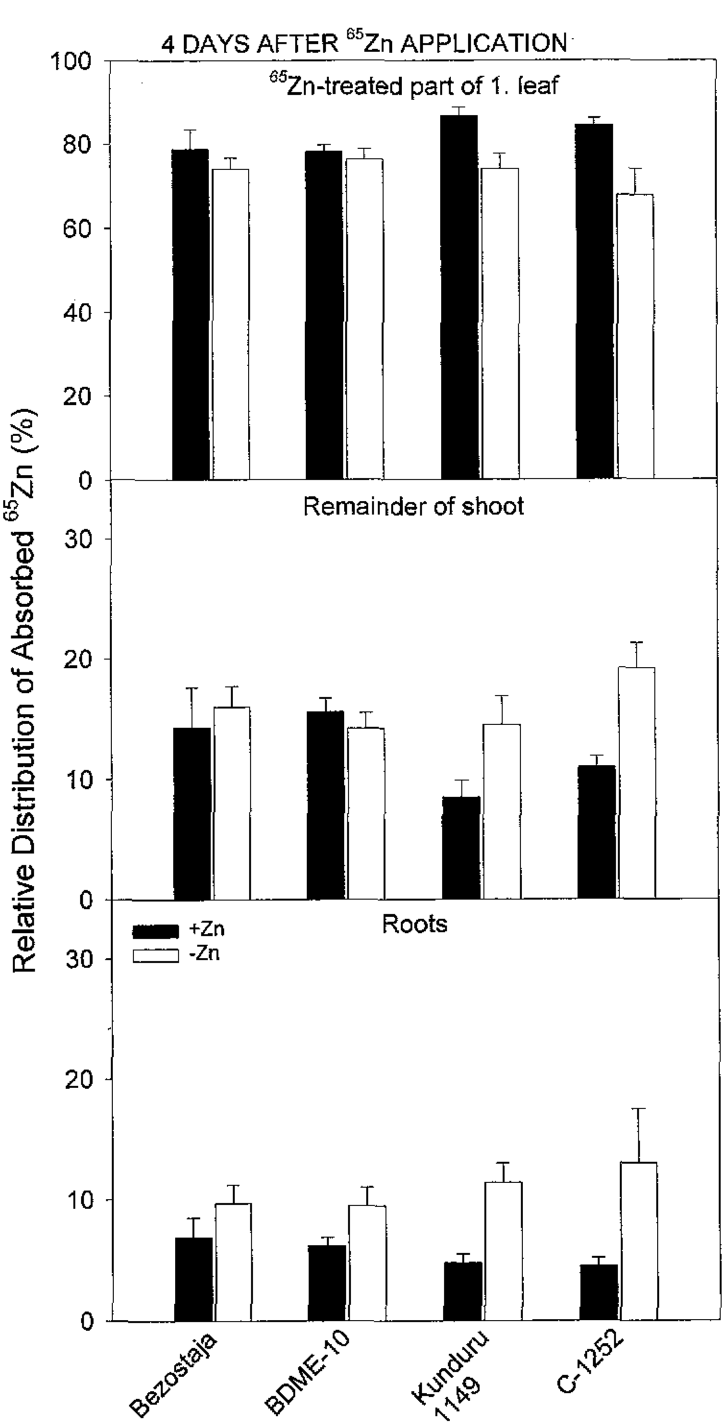

Figure 8.3. Relative distribution of absorbed ^{65}Zn after removal of extrasymplastic Zn (percentage of total activity of ^{65}Zn per plant part) in the treated part of the oldest leaf, remainder of shoot, and roots, 4 days after application of ^{65}Zn to the upper part (3 cm) of the first (oldest) leaf of bread wheat (Bezostaja and BDME-10) and durum wheat (Kunduru-1149 and C-1252) genotypes differing in Zn efficiency grown in nutrient solution. Data represent means±SD of four independent replicates

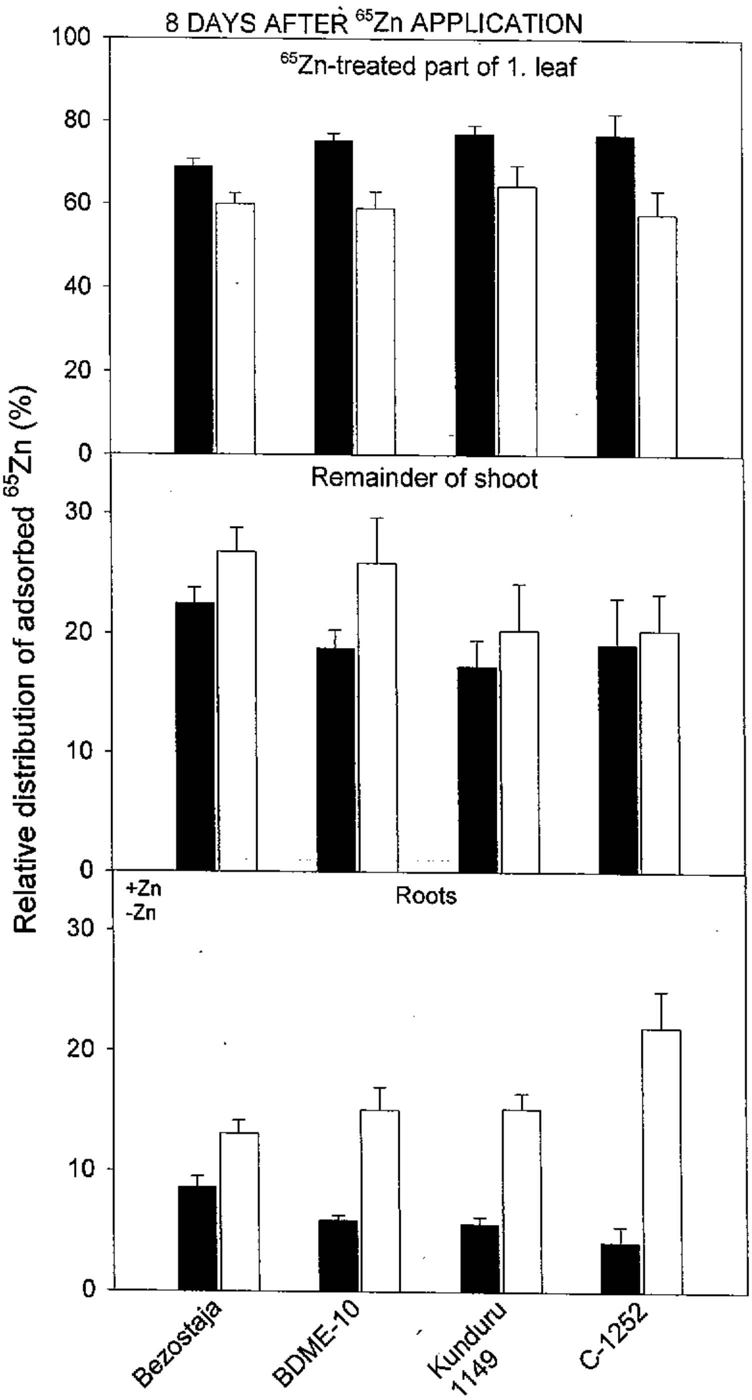

Figure 8.4. Relative distribution of absorbed ^{65}Zn after removal of extrasymplastic Zn (percentage of total activity of ^{65}Zn per plant part) in the treated part of the oldest leaf, remainder of shoot, and roots, 8 days after application of ^{65}Zn to the upper part (3 cm) of the first (oldest) leaf of bread wheat (Bezostaja and BDME-10) and durum wheat (Kunduru-1149 and C-1252) genotypes differing in Zn efficiency grown in nutrient solution. Data represent means±SD of four independent replicates

Also, wheat cultivars with different Zn efficiency did not differ in total Zn uptake (*Figure 8.2*). Although total uptake of foliar applied ^{65}Zn was not significantly affected by Zn nutritional status (+/-Zn preculture), relative distribution of total absorbed ^{65}Zn within plants was, however, clearly affected by Zn supply during preculture, particularly, 8 days after application of ^{65}Zn (*Figure 8.3* and *Figure 8.4*). Eight days after ^{65}Zn application, Zn-deficient and Zn-sufficient cultivars transported about 40 % and 26 % of the total absorbed Zn out of the applied area, respectively. Bread wheat cultivars with differential Zn efficiency did not differ in distribution of ^{65}Zn between shoot and roots under Zn deficiency. In all cultivars, the proportion of translocated ^{65}Zn was higher in shoots than roots, particularly in the case of plants supplied adequately with Zn. Compared with the bread wheats, the durum wheats tended to accumulate more ^{65}Zn in the roots and less in the remainder of shoot after 8 days of ^{65}Zn application (*Figure 8.4*). Under Zn-deficient conditions, the ratio of the Zn translocated into remainder of shoot to that in the roots after 8 days of ^{65}Zn application was about 1.9 for bread wheats and 1.1 for durum wheats. These ratios were much higher under Zn-sufficient conditions.

8.5. Discussion

Results of the present study show that Zn applied to old leaves can easily be translocated to young parts of shoots and roots under both Zn-deficient and Zn-sufficient conditions, indicating that phloem mobility of Zn is not limited in wheat. These results are in good agreement with the results obtained with wheat (Herren and Feller, 1994; 1997; Erenoglu et al., 2001), bean (Bukovac and Wittwer, 1957; Ferrandon and Chamel, 1988), and citrus (Wallihan and Heymann-Herschberg, 1956). In the case of citrus and bean plants, up to 80 % of the applied Zn was absorbed and translocated out of the treated leaves. However, in the studies with pistachio seedlings Zhang and Brown (1999) showed that only 5.4 % of the absorbed Zn by the leaves was translocated into other parts of seedlings. Similarly, in balsam fir seedlings more than 10 % of the needle-applied ^{65}Zn could be translocated into youngest needles (Lin et al., 1995). However, in spruce seedlings needle-applied ^{65}Zn could not be translocated from the treated leaf (Watmough et al., 1999) and in pecan plants only 0.2 % of the total applied Zn could be absorbed and exported out of treated leaves (Wadsworth, 1970). These results indicate that depending on plant species and experimental conditions, uptake and translocation of foliar applied Zn can greatly vary.

The amount of ^{65}Zn absorbed by leaves was not affected by Zn nutritonal status of plants when Zn was applied to leaf strips (*Figure 8.1*) or on to leaves of intact plants (Figure 2). However, translocation of leaf-applied Zn out of the treated leaf was strongly affected by the Zn nutritional status of plants. About 40 % and 25 % of total ^{65}Zn absorption were translocated from the treated leaves under Zn deficiency and adequate Zn supply, respectively (*Figure 8.3*; *Figure 8.4*). Similarly, Pearson and Rengel (1994) showed that in wheat remobilization of Zn from old leaves into generative organs is much greater under low supply than adequate supply of Zn. By contrast, Longnecker and Robson (1993, and references therein) reported that remobilization of Zn from older into younger tissues is greater with adequate than deficient Zn supply. According to Marschner (1995), lower remobilization and phloem transport of Zn from leaves of plants with low Zn supply is possibly related to greater amount of Zn firmly bound to cell constituents. As reported by Longnecker and Robson (1993) and by Marschner (1995), most of the studies dealing with Zn retranslocation from old leaves into young tissues were realized with old leaves showing senescence. The results presented here demonstrate that Zn can also be translocated out of leaves which were not senescent, which is in agreement with studies on Zn mobility in young rice seedlings (Hajiboland et al., 2001).

Differences in Zn efficiency among and within bread and durum wheats did not affect neither uptake nor translocation of leaf-applied Zn (*Figure 8.2; Figure 8.3; Figure 8.4*). However, irrespective of Zn efficiency bread wheat compared to durum wheats directed greater proportion of the translocated ^{65}Zn into young parts of shoots. These findings are in well accordance with the our recent results (Erenoglu et al., 2001). In that study, we observed that two bread wheats (Bezostaja; Efficient and BDME-10; Inefficient) and one bread wheat (Kunduru-1149; Inefficient) used in present study also did not show any clear relationship between Zn efficiency and retranslocation of pre-loaded ^{65}Zn. Similarly, in uptake experiments with supply of ^{65}Zn to roots it has been found that under Zn deficiency bread wheats translocate more Zn from roots into shoots than durum wheats (Rengel and Graham, 1995b; Cakmak et al., 1996b; 1998). It might be possible that Zn present in roots is more readily sequestered in the cell walls and/or vacuoles in durum than bread wheats, causing a larger retention of the translocated Zn in roots of durum wheats. This is particularly pronounced in C-1252 that is the most sensitive durum wheat genotype tested under severe Zn deficient soils in Central Anatolia (Cakmak et al., 1996a, 1997). However, the differences in Zn retention in roots of Zn-efficient and Zn-inefficient bread wheats and Zn-inefficient durum wheat Kunduru were very small. It appears that reduced translocation of Zn within plants, i.e., from roots into shoots does not generally contribute to the differences in Zn efficiency. Differences in Zn translocation within the plants were assumed as one major mechanism affecting expression of high Zn efficiency (Graham and Rengel, 1993; Cakmak et al., 1996b; Torun et al., 2000).

The results of the present work demonstrate that Zn is easily translocated from old into young leaves and roots via phloem in wheat, particularly under Zn deficiency. This indicates that foliar application of Zn can greatly improve nutritional status of wheat plants suffering from deficiency. Our results also indicate that the distribution of Zn between roots and shoots from foliar applied sources is not an important contributing factor in genotypic variation in Zn efficiency of wheat, particularly among bread wheats. Alternatively, differences in subcellular compartementation of Zn might be a relevant factor in expression of a high Zn efficiency. This aspect needs to be clarified in future studies.

9. Retranslocation of zinc (^{65}Zn) from older leaves to younger leaves and roots in wheat cultivars differing in zinc efficiency

9.1. Abstract

Retranslocation of zinc (Zn) from old leaves to young growing parts (young leaves and roots) was studied in a nutrient solution culture experiment using intact plants of two bread wheat (*Triticum aestivum* cvs. Bezostaja and BDME-10) and one durum wheat (*Triticum durum* cv. Kunduru-1149) cultivars differing in Zn efficiency. Plants were precultured for 6 days in a Zn free nutrient solution and, thereafter, loaded with 1 mmol m^{-3} $^{65}ZnSO_4$ during 3 days. Afterwards the roots were washed and the ^{65}Zn-loaded plants were transferred to ^{65}Zn free nutrient solution after washing the roots to remove surface bounded and extraplasmatic ^{65}Zn, an initial harvest was conducted. After ^{65}Zn pre-loading, plants were grown either with (1 mmol m^{-3} $ZnSO_4$) or without (0 mmol m^{-3} $ZnSO_4$) Zn supply. Three, 6, and 9 days after initial harvest, further plants were harvested. During the 9 days growth after ^{65}Zn pre-loading, dry weights of plants were not affected by the different Zn supply, with exception of durum wheat cv. Kunduru-1149. Already after the second harvest, young leaves of this cultivar without a further Zn supply had less dry matter than with Zn supply. Between day 3 (II. Harvest) and day 9 (IV. Harvest), up to 35% of the total ^{65}Zn present in 1., 2., and 3. leaves (older leaves) was moved to other parts of plant. The obtained data show that Zn can be retranslocated from old leaves of different wheat species to young leaves and roots via phloem. Further, this mobility is related to the Zn nutritional status of plants. Under low Zn supply, plants retranslocated more

Zn compared with plants supplied with adequate Zn. For example, under low Zn supply, 26-35% of total Zn content in older leaves was retranslocated, but only 14-18% under adequate Zn supply. However, no significant relationship between Zn efficiency and Zn retranslocation could be found in the studied wheat genotypes.

9.2. Introduction

Plant species and cultivars within a species show great differences in ability to take Zn up from soil and to utilise Zn internally. Wheat is a particular cereal showing great genotypical differences in Zn efficiency. In very severe Zn deficient calcareous soils of South Australia (Graham et al., 1992) and Turkey (Cakmak et al., 1996a), very sharp differences in Zn efficiency were observed between bread and durum wheat and also among cultivars of bread wheat.

Several physiological mechanisms which were discussed as possible reasons for differential Zn efficiency among and within bread and durum wheats, have been investigated, including differences in root morphology (Dong et al., 1995), release of Zn-mobilising phytosiderophores by roots (*Chapter 5*; *Chapter 6*; Cakmak et al., 1996b; Erenoğlu et al., 1996), internal utilisation of Zn (Rengel, 1995; Cakmak et al., 1997), root uptake and root-to-shoot translocation of Zn (*Chapter 7*; Rengel and Graham, 1996; Cakmak et al., 1998; Erenoglu et al., 1999) and translocation of foliar applied Zn to the other parts of seedlings (*Chapter 8*; Erenoglu et al., 2000).

Transpration and root pressure are the main forces for the root-to-shoot transport of mineral nutrients via xylem vessels. In spite of this, rapidly-growing and simultaneously low transpiring organs such as young leaves have to be supplied with mineral nutrients mainly by phloem import. Long distance movement in the phloem happens in living cells, sieve tubes (Marschner, 1995). The principles of the phloem transport mechanisms were proposed in a pressure flow hypothesis based on the principle of the osmometer (Münch, 1930).

The remobilisation of nutrients from one part of the shoot and the transport of these via the phloem to other parts of shoot or another organs is a very important phenomena in plant nutrition. According to Marschner (1995), a decrease in the net content of a mineral nutrient per organ is named as "remobilisation". The ability of plants to remobilise the nutrients changes from one nutrient to another. Deficiency symptoms of nutrients with high phloem mobility are mainly observed in older leaves. Nutrients such as N, P, and K are easily transported to other plant organs from leaves in which they have been deposited during an earlier growth stage. Calcium and B are either phloem immobile, or have extremely low or no retranslocation, are accumulated in old leaves. Some others such as S, Fe, Cu, Zn, Mn, and Mo have an intermediate mobility (Bucovac & Wittwer, 1957) which is dependent on the plant species (Epstein, 1971) or developing stage of the plant (Loneragan et al., 1976).

The phloem mobility of Fe has already been shown by foliar application of radio-labelled Fe (Bucovac & Wittwer, 1957) and by analysing the composition of phloem sap (Hocking, 1980). Although Fe is phloem mobile, typical deficiency symptoms (chlorosis) are observed in young leaves under Fe deficiency whereas mature leaves remain green (Bergmann, 1988). It has been reported that in Fe-deficient plants, only a small quantity of Fe from leaves can be remobilised to other organs (Pandey & Kannan, 1979). Remobilisation and retranslocation of only a certain fraction of total Fe in mature leaves were reported, irrespective of nutritional status of plants (Zhang, 1996).

The movement of foliar applied Cu to other parts of the plant has also been shown (Bukovac & Wittwer, 1957). Up to now, Zn was known as one of the intermediate mobile nutrients in phloem (Bukovac & Wittwer, 1957;

Marschner, 1995). Zinc mobility in phloem did not attract very much the researchers in the past years. Only limited number of investigations in Zn mobility were done very intensively in the 50's (Wood and Sibly, 1950; Williams and Moore, 1952; Bukovac and Wittwer, 1957; Riceman and Jones, 1958). Recently, Pearson and Rengel (1994) showed that wheat plant had very high Zn remobilization from old leaves to generative organs under Zn deficiency than at adequate Zn supply. But these recent results contradict the results published in the 50's. The results in the 50's pointed out that the total quantity of mobilised Zn from old leaves to growing young parts of shoot, flowers and seeds of plants depended on the Zn amount in old organs. Plant species supplied at luxury with Zn could remobilise enough Zn to be detected from old leaves to young parts of plants. But at deficient supply of Zn, remobilization of Zn was very low for the same species. Recently, for wheat species (bread and durum wheat) it has been shown that the plants supplied with a deficient level of Zn re-translocated more Zn from ^{65}Zn applied part of the oldest leaf to young leaves and roots (Chapter 8; Erenoglu et al., 2001). Similar observations have been also done for rice by Hajiboland and her co-workers (2001).

The remobilisation of a given nutrient varies greatly from species to species (Loneragan et al., 1976). As an increasing interest in selection and breeding of genotypes with high nutrient efficiency, identification of genotypes with high remobilisation capacities from older to younger leaves is considered important (Marschner, 1995).

The present study is aimed on (1) study the remobilization of Zn from old leaves to young growing points (young leaves and roots) during the vegetative growth phase with intact plants to evaluate the effect of de-rooting and reproductive stage-induced senescence and (2) test the relationship between the remobilization of ^{65}Zn, the distribution of this remobilized Zn and the differential Zn efficiency of cultivars used in this research.

9.3. Materials and Methods

Two bread wheat (*Triticum aestivum* L., cvs. Bezostaja and BDME-10) and one durum wheat (*Triticum durum* L., cv. Kunduru-1149) cultivars were used in the experiment. According to Kalayci et al., (1999), these cultivars differ in Zn efficiency when they are grown in a Zn deficient soil. Among them, while the bread wheat cv. Bezostaja is Zn-efficient and the others are -ineffcient. Plants were grown in a nutrient solution under controlled climatic conditions (light/dark regimes of 16/8 h, temperature 24/20 ^{o}C and a photosynthetic photon flux density of 350 $\mu mol\ m^{-2}\ s^{-1}$ at plant height). After germination in quartz sand moistened with saturated $CaSO_4$ solution, seedlings were transferred to 2.5-L plastic vessels containing the continuously aerated nutrient solution (for the composition of nutrient solution see *Chapter 5*).

Plants were grown in Zn-free nutrient solution for 6 days. During this period nutrient solution was changed once on 3. day. Thereafter, the plants were supplied with 1 mmol m^{-3} $ZnSO_4$ labelled with ^{65}Zn in a 1.2 L plastic vessels containing four bundles with 2 plants for 3 days. After pre-loading, the plants were taken out from ^{65}Zn containing nutrient solution and solution sampling were done to determine the ^{65}Zn deplition rates by each cultivar. Then, roots were washed with 1 mol m^{-3} $CaSO_4$ and 1 mol m^{-3} Na-EDTA to remove surface bounded and extraplasmatic Zn and transferred into ^{65}Zn-free nutrient solution. After the washing of roots, one bundle of each pot was harvested and whole plant was divided into five parts: i) first leaf, ii) second leaf, iii) third leaf, iv) remainder shoot, and v) roots. After that, plants were grown either with (+Zn: 1 mmol m^{-3} $ZnSO_4$) or without (-Zn) Zn supply. During 9 days growth after pre-loading, plants harvested another 3 times (3, 6, and 9 days after initial harvest) as described above. The harvested samples were dried at 65 ^{o}C, weighed, and ashed at 500 ^{o}C. Later on, ashed samples were dissolved in 1% HCl and the activity of ^{65}Zn in samples was measured by Liquid Scintillation Counter.

9.4. Results

Analysis of samples taken from uptake solution after the pre-loading of ^{65}Zn depletion showed that the cultivars did not differ in total ^{65}Zn uptake during pre-loading of ^{65}Zn (data not shown). These findings are also supported by similarities in the absolute values of total ^{65}Zn in wheat cultivars after washing the roots with $CaSO_4$ and Na-EDTA to have ^{65}Zn-free root surface and extraplasma (*Table 9.1.*).

Table 9.1. Total amount of accumulated Zn (whole plant) in bread (Bezostaja and BDME-10) and durum wheat (Kunduru-1149) cultivars during 3 days pre-loading with 1 mmol m^{-3} $^{65}ZnSO_4$ and after removing surface bounded and extraplasmatic ^{65}Zn

Genotypes	Total absolute amount of Zn (nmol plant-1)	
	-Zn	+Zn
T. aestivum		
Bezostaja	147	151
BDME-10	154	152
T. durum		
Kunduru-1149	170	151

Relative ^{65}Zn amount in primary leaves of bread and durum wheat cultivars grown for 9 days with and without Zn application after pre-loading of ^{65}Zn is presented in *Figures 9.1, 9.2,* and *9.3* together with dry weights. In the first three harvest period, there was no ^{65}Zn movement from the first leaf to the other parts of plant in all wheat cultivars with further Zn application after pre-loading of ^{65}Zn (*Figure 9.1*). However, at the fourth harvest, particularly the plants supplied with no Zn started to move ^{65}Zn from the first leaf to the other plant organs (*Figure 9.1*). Since there was a continuum of ^{65}Zn transport from roots to shoot, particularly to 2. and 3. leaves, no ^{65}Zn retranslocation could be observed during three days after pre-loading (*Figure 9.2*). After the second harvest, ^{65}Zn movement out of these 2. and 3. primary leaves of the Zn deficient plants has been started. At this harvest period, there was no ^{65}Zn retranslocation from these leaves to the other parts of the plants supplied with further Zn after pre-loading of ^{65}Zn (*Figure 9.2*). Comparison of the total ^{65}Zn amount in the primary leaves (1., 2., and 3. leaves) of wheat cultivars used in this study at the second and the fourth harvests showed that up to 35% of total ^{65}Zn was transported from the primary leaves to the other parts of all cultivars (*Figure 9.3*). At the fourth harvest, also in primary leaves of Zn sufficient plants, less ^{65}Zn was found than at the third harvest, indicating retranslocation of ^{65}Zn from these parts of plants (*Figure 9.3*).

Relative ^{65}Zn amount in remainder parts of shoot (younger leaves) and dry weight of these parts in bread and durum wheat cultivars grown for 9 days with and without Zn application after pre-loading of ^{65}Zn are given in *Figure 9.4*. In all cultivars, the relative ^{65}Zn amount in younger leaves increased from the first harvest to the fourth one at both Zn nutritional status (*Figure 9.4*). Nevertheless, this increment was lower in the durum wheat cv. Kunduru-1149 than the bread wheat cvs. Bezostaja and BDME-10 (*Figure 9.4*). Both bread wheat cultivars tended to load younger parts with more ^{65}Zn under deficient Zn supply compared to the plants supplied with sufficient Zn after pre-loading period of ^{65}Zn (*Figure 9.4*).

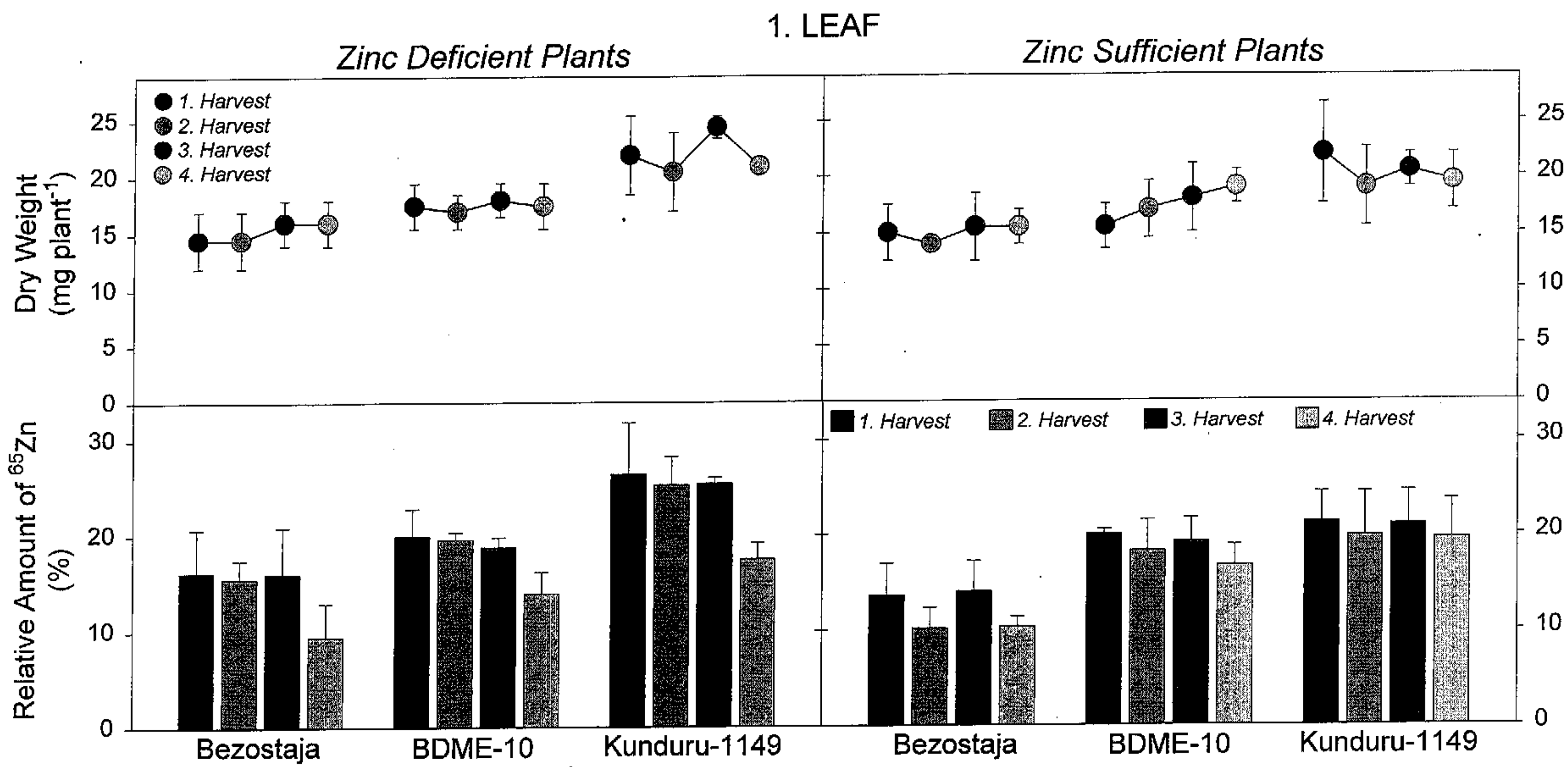

Figure 9.1. Dry weight and relative ^{65}Zn amount in 1. leaf of bread (Bezostaja and BDME-10) and durum wheat (Kunduru-1149) cultivars differing in Zn efficiency grown for 9 days in nutrient solution with or without further Zn supply after pre-loading of 1 mmol m^{-3} $^{65}ZnSO_4$. Data represents means±SD of four independent replicates

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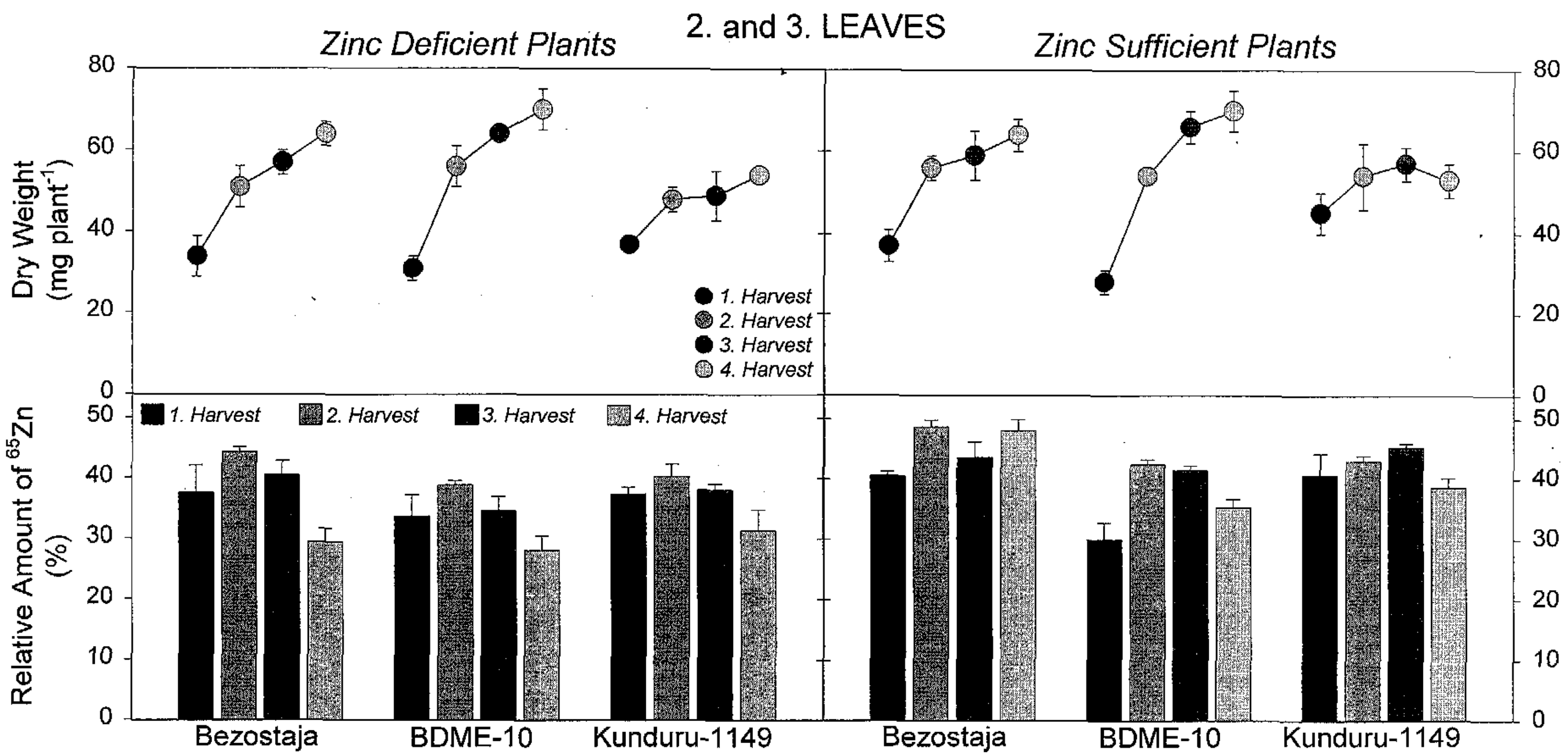

Figure 9.2. Dry weight and relative ^{65}Zn amount in 2. and 3. leaves of bread (Bezostaja and BDME-10) and durum wheat (Kunduru-1149) cultivars differing in Zn efficiency grown for 9 days in nutrient solution with or without further Zn supply after pre-loading of 1 mmol m^{-3} $^{65}ZnSO_4$. Data represents means±SD of four independent replicates

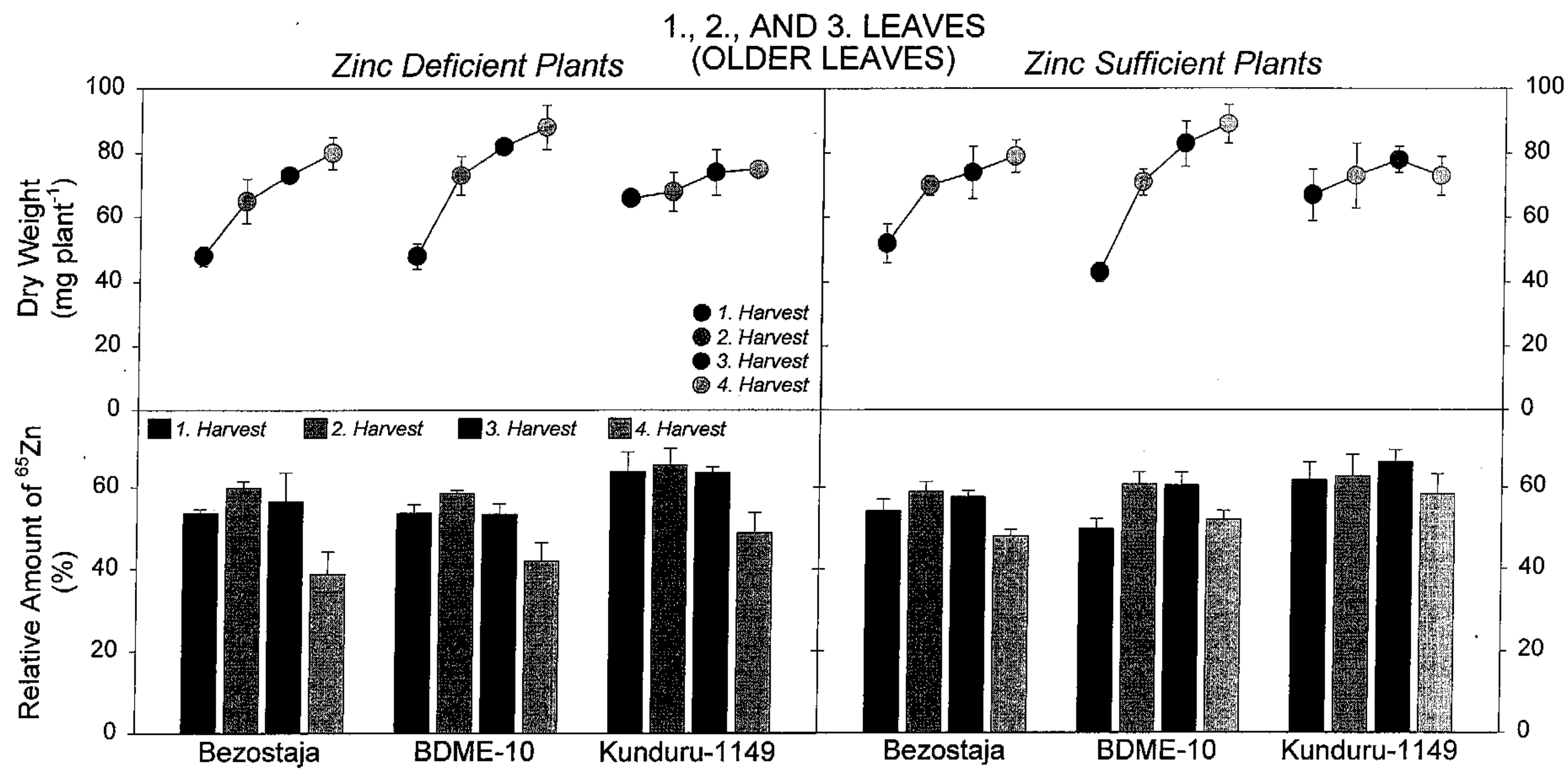

Figure 9.3. Dry weight and relative ^{65}Zn amount in 1., 2., and 3. leaves (older leaves) of bread (Bezostaja and BDME-10) and durum wheat (Kunduru-1149) cultivars differing in Zn efficiency grown for 9 days in nutrient solution with or without further Zn supply after pre-loading of 1 mmol m^{-3} $^{65}ZnSO_4$. Data represents means±SD of four independent replicates

The data presented in *Figure 9.5* shows the relative ^{65}Zn amount in roots of bread and durum wheat cultivars grown for 9 days with and without Zn application after pre-loading of ^{65}Zn. Up to the fourth harvest, the amount of ^{65}Zn in the roots of all cultivars at both Zn levels was reduced (*Figure 9.5*). However, at the fourth harvest, the cultivars again started to accumulate ^{65}Zn in roots. But this increase was higher in the plants with no Zn supply after pre-loading of ^{65}Zn than in the plants supplied with sufficient Zn (*Figure 9.5*).

During 6 days growth after pre-loading of $^{65}ZnSO_4$, neither shoot nor root growth of cultivars were affected by Zn supply with the exception of younger leaves (remainder parts of shoot) of durum wheat cv. Kunduru-1149 (*Figure 9.4*). Already at the third harvest (6 days after pre-loading of $^{65}ZnSO_4$), the Zn-deficient durum wheat had less dry matter production of remainder parts of shoot than Zn-sufficient one. In the same period, the root growth of the same cultivar did not change (*Figure 9.5*). At the fourth harvest, while the growth of younger leaves of the bread wheat cvs. Bezostaja and BDME-10 was not influenced much by differential Zn supply, the durum wheat cv. Kunduru-1149 at the third harvest had less dry matter of young leaves under low Zn supply (*Figure 9.4*). On the day of the fourth harvest, the root growth of all cultivars tended to increase at deficient Zn treatment, particularly, in the durum wheat cv. Kunduru-1149 (*Figure 9.5*). This increased root growth causing a lower shoot/root dry weight ratios in plants supplied with low Zn is a widely known response of wheats to Zn deficiency (Cakmak et al., 1996a; Erenoglu et al., 2000; Rengel and Graham, 1995a).

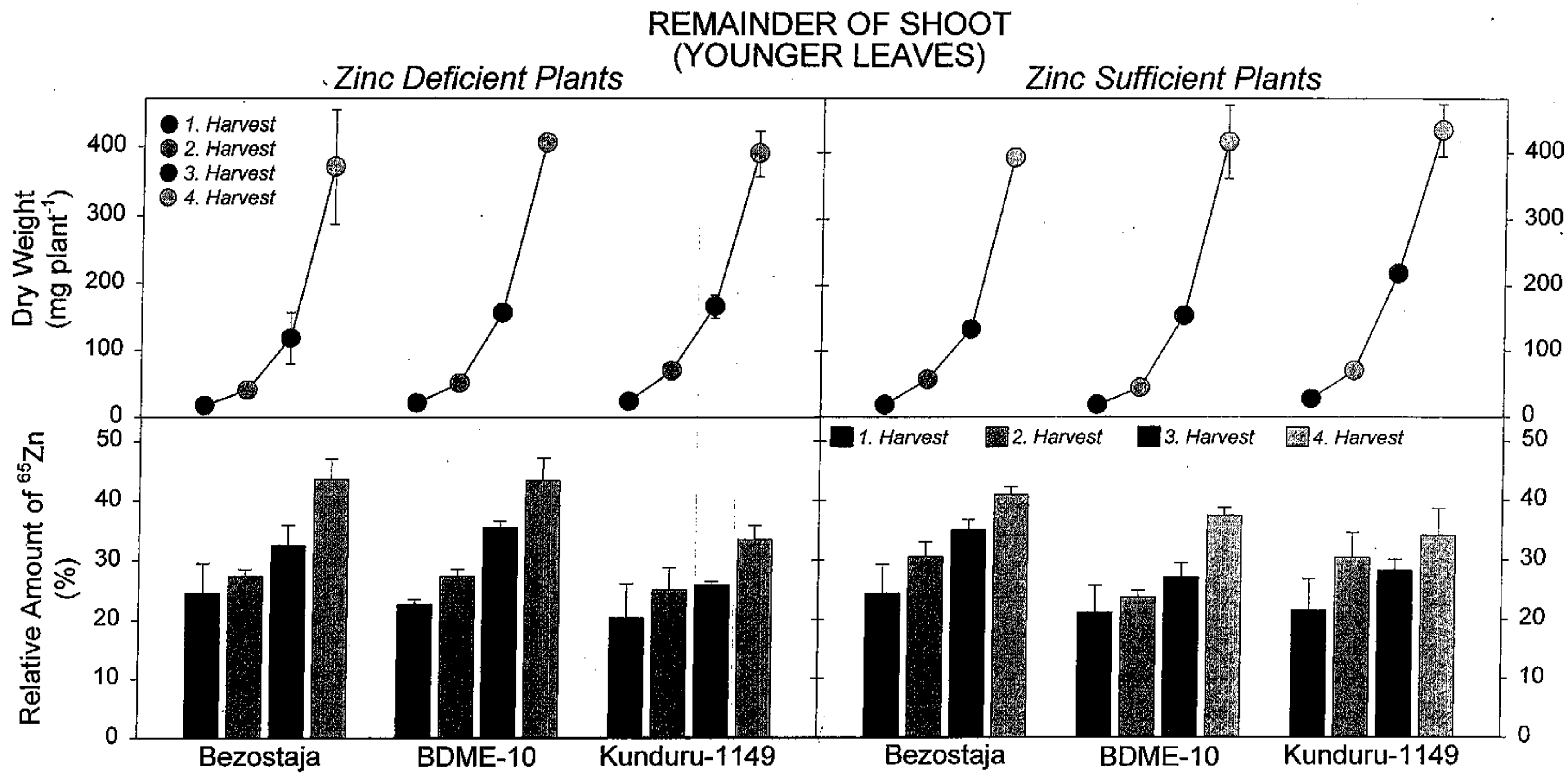

Figure 9.4. Dry weight and relative ^{65}Zn amount in remainder parts of shoot (younger leaves) of bread (Bezostaja and BDME-10) and durum wheat (Kunduru-1149) cultivars differing in Zn efficiency grown for 9 days in nutrient solution with or without further Zn supply after pre-loading of 1 mmol m^{-3} $^{65}ZnSO_4$. Data represents means±SD of four independent replicates

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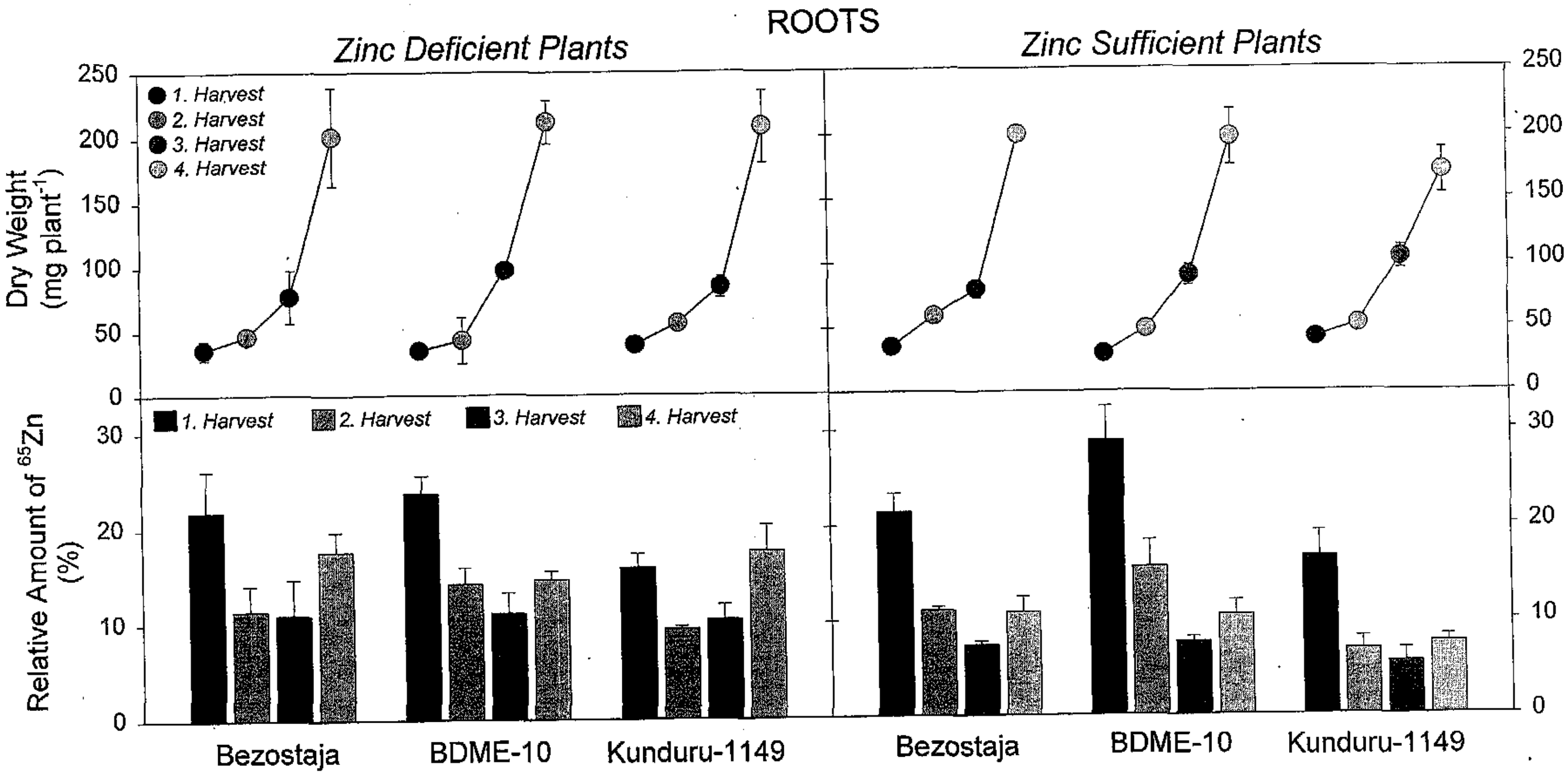

Figure 9.5. Dry weight and relative ^{65}Zn amount in roots of bread (Bezostaja and BDME-10) and durum wheat (Kunduru-1149) cultivars differing in Zn efficiency grown for 9 days in nutrient solution with or without further Zn supply after pre-loading of 1 mmol m^{-3} $^{65}ZnSO_4$. Data represents means±SD of four independent replicates

9.5. Discussion

Results of this chapter show the clear retranslocation of Zn from the primary leaves (1., 2., and 3. leaves) to the other parts (younger leaves and roots) of wheat via the phloem under both Zn deficient and sufficient conditions during a longer growth period with many harvests. These results are in good agreement with the results presented in *Chapter 8* of this PhD thesis and also with the results obtained with wheat (Herren & Feller, 1994; 1997), bean (Bukovac & Wittwer, 1957; Ferrandon & Chamel, 1988), citrus (Wallihan & Heymann-Herschberg, 1956), and pistachio (Zhang & Brown, 1999). In all studies mentioned above, Zn was applied on leaves as radio-labelled Zn (^{65}Zn), thereafter, ^{65}Zn measurement was made in other parts of plants. In each experiment, different relative amount of ^{65}Zn could be monitored in the different parts of plants showing that uptake and translocation of foliar applied Zn can greatly vary. This variation can be dependent on plant species and experimental conditions.

In the literature, there are contradictory results about the effect of the adequacy of supply of the nutrient. Some researchers suggested that the nutrients have higher mobility at luxury supply and a lower mobility at lower or insufficient supply (Riceman & Jones, 1958; Loneregan et al., 1976). In contrast, Pearson and Rengel (1994) showed that the wheat had a higher Zn remobilization from old leaves to generative organs under deficient Zn supply than at adequate Zn supply. Also, the results obtained by monitoring the movement of foliar applied ^{65}Zn from the oldest leaf to the other parts of the plant in *Chapter 8* show the same tendency which under low Zn supply, more Zn is transported via the phloem than under adequate Zn supply. Similar results from rice plants in which the rate of Zn remobilisation depends on Zn supply, were also shown (Hajiboland et al., 2001). However, no differences were obtained in the remobilization of Fe from primary leaves to the shoot apex in bean plants with different Fe nutritional status (Zhang et al., 1996a, b).

The plant genotypes could show genotypical differences in the remobilization of Zn from the older leaves to young growing parts (young leaves and roots) of the plant as in Zn efficient and inefficient rice cultivars (Hajiboland et al., 2001). Besides, the role of an efficient Ca remobilisation and retranslocation from older leaves into the youngest tissue in expression of a high nutrient use efficiency has also shown in Ca-efficient cowpea genotypes (Horst et al., 1992). As seen in the results of the present chapter, the wheat genotypes did not have so much differences in the retranslocation of ^{65}Zn from the older leaves to the younger leaves and the roots. When the second and third leaves are considered (*Figure 9.2*), it can be seen that only bread wheat cvs., Bezostaja and BDME-10, although they increased the dry weight of the oldest three leaves more than the durum wheat cv. Kunduru-1149 during 9 days growth, they tended to remobilise more Zn from these leaves to the other parts of plant.

As reviewed by Longnecker and Robson (1993), most of the scientific reports on Zn remobilization are concentrated on senescence-induced remobilization, at the beginning of the change from vegetative to generative period or during generative growth (Williams and Moore, 1952; Riceman and Jones, 1958; Herren and Feller, 1994). The close relation of the extent of remobilisation of Zn with leaf senescence (Marschner, 1995) and with N deficiency (Ramani & Kannan, 1985) was also shown. However, results presented here (*Figure 4.2*) and results obtained by Hajiboland et al. (2001), respectively for wheat and rice, have pointed out that remobilization could also occur in the leaves which are not in senescence. The decrease in total Zn amount of 2. and 3. leaves (still growing leaves, particularly bread wheat cultivars) from second to fourth harvest (*Figure 4.2*) leads to suggestion that Zn can be moved out of leaves which are still a sink for assimilates. In agreement with the results given here, using rice plants Obata and Kitigashi (1982) showed that Zn can be retranslocated out from leaves as soon as elongation ceased but before the dry weight of leaves reached its maximum.

It is difficult to comment on the distribution of ^{65}Zn retranslocated out of the first three leaves, since there was still ^{65}Zn movement from roots to shoot, particularly during 6 days growth after pre-loading (*Figure 9.5*). However, in general, it could be mentioned that all cultivars with the deficient supply of Zn remobilized more ^{65}Zn to roots than with the sufficient supply of Zn (*Figure 9.5*). By taking the changes in the total content of ^{65}Zn of roots and younger leaves from the first to the forth harvest periods into consideration, it can be done some superficial interpretations on the redistribution of the remobilized ^{65}Zn, since, supposedly the transport of ^{65}Zn from the roots to the shoot has already been completed. In such a manner that, during the period between the third and the last harvest, in all genotypes, almost the similar amounts of ^{65}Zn were moved from old leaves to the roots and the young leaves (*Figures 9.4 and 9.5*).

It can be concluded that Zn is phloem mobile nutrient in wheat species. However, there is no genotypical differences among and within the wheat species in remobilization, retranslocation, and redistribution of Zn which had been tested as possible reasons of differential Zn efficiency of cereals.

10. Final Discussion

Zinc deficiency is one of the most common micronutrient deficiencies in cereals, especially in wheat, limiting significantly grain yield, for example in Australia (Graham et al., 1992), India (Takkar and Walker, 1993), and Turkey (Cakmak et al., 1996a; 1999a). According to White and Zasoski (1999) the extent of low Zn availability in soils is comparable with the extent of many macronutrient deficiencies causing large economical losses.

The use of fertilizers to eliminate the problem of Zn deficiency is an often used practice. However, since crop species show differential susceptibility to Zn deficiency (Takkar, 1991; Tiwari& Dwivedi, 1990), the selection and breeding of plant genotypes that have high capacity to acquire Zn from soils or use more efficiently Zn absorbed by their roots, appear as an alternative method to circumvent Zn deficiency problems in cereals. Differential Zn efficiency among various genotypes has been reported for potato (Sharma & Grewal, 1990), navy-bean (Jolly & Brown, 1991), soybean (Saxena & Chandel, 1992), and maize (Ramani & Kannan, 1985).

There are substantial differences in susceptibility to Zn deficiency between cereal species and also between cultivars of a given cereal species. Therefore, one rye, two bread wheat, and one durum wheat cultivars were grown on a soil with low Zn availability to test their growth performances. In the study presented here, rye clearly showed the best growth, highest Zn efficiency, (expressed as the ratio of shoot dry matter produced under Zn deficiency compared to adequate Zn nutrition). Durum wheat had the strongest growth reduction under Zn deficiency. After 30 days of growth in the greenhouse, Zn efficiency of cereals were 77% for rye, 58% for bread wheat cv. Bezostaja, 49% for bread wheat cv. BDME-10, and 47% for durum wheat cv. Kunduru-1149 (*Chapter 3*). These results were in full-agreement with the earlier observations conducted with cereals (Cakmak et al., 1997; Ekiz et al., 1998). The resistance of cereals to Zn deficiency was found to increase in the order: durum wheat<oat≤bread wheat<barley<triticale<rye (Cakmak et al., 1997; Ekiz et al., 1998). Also diploid (AA), tetraploid (BBAA) and hexaploid (BBAADD) wheat cultivars differ greatly in their Zn efficiency (i.e., superior growth and yield under Zn-deficient conditions relative to other cultivars). Thus, among the wheat genomes, the A genome exerts a stimulative effect while B genome exerts a repressive effect on the expression of high Zn efficiency (Cakmak et al., 1999b, c).

Similar to growth under Fe deficiency, graminaceous species also release PS's of the mugineic acid family from roots when grown under Zn deficiency. These PS's have been shown to be involved in the mobilization and uptake of Zn from the soil (Zhang et al., 1989, 1991). In subsequent studies, differences in Zn efficiency between durum and bread wheat genotypes were related to differences in the release of PS's (Cakmak et al., 1994, 1996c; Walter et al., 1994). In a study conducted by using six bread wheat and four durum wheat cultivars, the higher sensitivity of durum wheat to Zn deficiency was attributed to its lower capacity to synthesize and release PS's, and moreover, differences in the release of PS's were also discussed as a suitable parameter for an evaluation of Zn efficiency among different cultivars of bread wheat (Cakmak et al., 1996c). In the present study, durum wheat which is the most sensitive cereal species to Zn deficiency secreted the lowest amount of PS's. This is in full agreement with earlier findings (Cakmak et al., 1994, 1996c; Walter et al., 1994). Rye, triticale, and bread wheat showed similar rates of PS's release although they differ in their resistance to Zn deficiency, irrespective of whether they were grown in the field or in the greenhouse (*Chapter 5* and *Chapter 6*). Thus, PS release is most probably not related to susceptibility of bread wheat, rye, and triticale cultivars to Zn deficiency. In case of barley which shows an intermediate susceptibility to Zn deficiency relative to triticale and bread wheat, a lower rate of PS release was found in a separate nutrient solution experiment (Erenoglu et al., 2000) compared to the PS release of triticale and bread wheat. This observation supports the idea that the capacity for PS release under Zn deficiency might explain the low Zn efficiency in durum wheat but not the differences in Zn efficiency among the other cereals.

In earlier studies regarding the root uptake for Zn, only extremely Zn-efficient and -inefficient bread and durum wheat cultivars, were used for comparison. Then, a close correlation between Zn efficiency and Zn uptake capacity by roots was observed (Rengel & Graham, 1996; Rengel et al., 1997). Genotypical differences in Zn uptake capacity were also found for rice (Bowen, 1986) and sorghum (Ramani & Kannan, 1985). However, with respect to Zn efficiency, the results obtained in those studies should be considered very consciously, because plants were supplied with unusually high Zn concentrations (up to 0.5 mol m^{-3}). Comparison of two bread wheat cultivars for their Zn uptake kinetics by using physiologically relevant concentrations of Zn in nutrient solution (0 to 2 mmol m^{-3}) has recently shown that the Zn-efficient bread wheat cultivar had only a 30% higher rate of net Zn uptake relative to the Zn-inefficient bread wheat (Rengel & Wheal, 1997). The results of the experiments conducted here, using one rye, three bread wheat, and one durum wheat genotypes showed that rye had the highest Zn uptake rate under low Zn nutritional stutus (*Chapter 7*), suggesting that a higher Zn uptake capacity should be considered as a likely additional physiological mechanism contributing to Zn efficiency in cereals. A superior efficiency for rye relative to other cereal species was also found in long-term field experiments by calculating Zn accumulation in shoots (Cakmak et al., 1997a; Ekiz et al., 1998). In good agreement with earlier studies (Cakmak et al., 1998; Rengel & Wheal, 1997; Rengel et al., 1998), a close relationship between the high sensitivity of durum wheat to Zn deficiency and a relatively low capacity to take up Zn under Zn-deficient conditions was found in the present investigations (*Chapter 7*). Although high and low Zn efficiency of rye and durum wheat, respectively, could be attributed to their differential abilities for Zn uptake from nutrient solution (*Chapter 7*; Cakmak et al., 1998; Erenoglu et al., 1999), differences in the sensitivity to Zn deficiency within bread wheat cultivars did not correlate with the uptake rate of Zn (*Chapter 7*). However, the reason for superior Zn uptake capacity of rye under Zn-deficient growth is not clear at present. In wheat, a high Zn uptake capacity was associated with the expression of a 34-kDa polypeptide in the root plasma membrane, and this polypeptide was discussed as a possible structural unit of a plasma membrane transporter for Zn (Rengel & Hawkesford, 1997; Rengel & Wheal, 1997). More recently, Zn transporter genes have been isolated from *Thlaspi caerulescens* a Zn hyperaccumulator (Pence et al., 2000). The results of that study on ZNT1 expression and high-affinity Zn^{2+} uptake in roots of *Thlaspi caerulescens* and in a related nonaccumulator, *Thlaspi arvense*, indicated that change in the regulation of ZNT1 gene expression by plant Zn status results in overexpression of this transporter and in enhanced Zn influx in roots of the hyperaccumulating *Thlaspi* species. Thus, overexpression of a Zn transporter could also be involved in the high Zn uptake capacity observed in rye.

Since Zn can also be absorbed by graminaceous species in the form of ZnPS (von Wiren et al., 1996), it might be possible that differential ZnPS uptake capacities act as physiological mechanisms to increase Zn efficiency. Preliminary results obtained from short-term uptake studies showed that there is no correlation between ZnPS uptake capacity and Zn efficiency in wheat (B. Singh, B. Erenoglu, & N. von Wiren, unpublished data).

Based on these results it has been suggested that Zn-efficient bread wheat and Zn-inefficient bread wheat and durum wheat cultivars may differ in the internal utilization and mobility of Zn, because of higher internal concentrations of PS's in Zn-efficient bread wheat (Cakmak et al., 1996c) and higher activities of Zn-containing enzymes such as superoxide dismutase and carbonic anhydrase, although Zn-efficient and -inefficient cultivars showed the same total Zn concentration in leaves (Rengel, 1995; 1999; Cakmak et al., 1997; Cakmak, 2000).

Based on, Torun et al. (2000) assumed that in Zn-efficient bread wheat, Zn could be retranslocated in larger amounts from older leaves to meristematic tissues allowing a better and continuous growth under inadequate supply of Zn. An efficient nutrient remobilisation and retranslocation from older leaves to the youngest tissue was described as an important factor for Ca efficiency in cowpea (Horst et al., 1992) and for Zn efficiency in rice (Hajiboland et al., 2001). To clarify the possible involvement of Zn retranslocation from older tissues to young growing tissues in wheat, the translocation of ^{65}Zn applied to tip of the oldest leaf was monitored. Eight days after application, up to 40% of absorbed Zn by the leaf was translocated into other organs of Zn-deficient plants, while in Zn-sufficient plants, only 25 % of ^{65}Zn moved out of the ^{65}Zn-applied area, although the absorption from leaf surface was as high as in Zn-deficient plants (*Chapter 8*). Thus, Zn must be considered as phloem-mobile or at least as moderately phloem-mobile which is in agreement with Marschner (1995), but not with (Orphanos, 1975). However, in contrast to the remobilisation of Ca in cowpea (Horst et al., 1992) and of Zn in rice (Hajiboland et al., 2001), the present results did not approve that wheat cultivars might differ in the translocation of foliar applied Zn (*Chapter 8*). There is no clear evidence that Zn applied on leaf surface is in the same compartment or binding form as internal Zn originating from the absorption by roots. Hence, it is probable that foliar applied Zn follows a different metabolic/physical pathway compared to internally translocated Zn. Therefore, two bread wheat and one durum wheat cultivars differing in Zn efficiency were preloaded with ^{65}Zn during preculture and Zn transport out of the oldest three leaves during 9 days after the preloding period was determined. During that period, up to 35% of the total ^{65}Zn present in the oldest three leaves had moved to other plant organs. While Zn-deficient plants retranslocated 26-35% of the labelled Zn out of the older leaves, in Zn-sufficient plants the corresponding value ranged between 14-18%. The wheat cultivars tended to retranslocate similar amounts of ^{65}Zn from older leaves to other plant organs, only in case of Zn-efficient bread wheat cv. Bezostaja was little higher (*Chapter 9*). These findings were in good agreement with the observations made in *Chapter 8*, suggesting that there is no obvious dependence of Zn efficiency on the retranslocation of Zn in wheat.

Vesicular-arbuscular (VA) mycorrhizae are widespread in soils, and often growth of VA-mycorrhizal plants is increased in comparison to nonmycorrhizal plants. This beneficial effect on plant growth has mainly been attributed to higher phosphorus (P) uptake and consequently better P nutrition of mycorrhized plants. The higher uptake of P by mycorrhized plants is mainly due to absorption and translocation of P from distant areas which are inaccessible to plant roots but exploited by hyphae (Sanders and Tinker, 1971, 1973; Viebrock, 1988). In addition to P, VA mycorrhizal fungi can also enhance the acquisition of Zn (Swaminathan and Verma, 1979; Gnekow and Marschner, 1989; Kothari et al., 1990, 1991). However, chickpea genotype, Weber et al. (1993) showed that "strongly mycorrhized" plants had lower Zn concentrations in the shoots than "weakly mycorrhized" plants and attributed this to a dilution of Zn in the tissue due to the increased growth of the mycorrhized plants. To available literature, there was so far no report on the influence of AM on Zn uptake and differential Zn efficiency of wheat. Only in a review (Rengel, 1999), it has been proposed that the role of AM in Zn uptake and differential Zn efficiency of wheat is negligible when wheat is grown on Zn-deficient sandy soils (e.g. of Australia), where a low mycorrhizal infection potential exists (Graham & Rengel, 1993) and mycorrhizal colonisation is strongly suppressed whenever P is supplied.

However, in case of wheat grown on Zn-deficient calcareous soils with heavy texture in Turkey, high mycorrhizal infection rates were determined for cereal roots, even the plants were fertilized with P (Ibrahim Ortas, personal communication). In contrast to most of the earlier findings (Swaminathan and Verma, 1979; Gnekow and Marschner, 1989; Kothari et al., 1990, 1991), the results in the *Chapter 4* of this thesis showed that under extremely low availability of Zn in calcareous soils with heavy texture, the presence of AM infection does not improve the Zn nutritional status of wheat. Instead, AM more likely reduced Zn uptake in wheat, irrespective of genotypical differences, possibly due to an altered carbohydrate metabolism. Among the Zn-dependent enzymes involved in carbohydrate metabolism, carbonic anhydrase (CA) plays an important role (Cakmak, 1988) since increasing Zn stress causes a sharp declines in CA activity (Ohki, 1976). This decline is the most prominent change in the activity of enzymes involved in carbohydrate metabolism (Shrotri et al., 1983). According to Uren (2001), the percentage of C secreted by root exudates relative to the total C fixed by soil-grown plants is less than 1%. If it is considered that rhizosphere processes, particularly the secretion of chelators, affect the mobilization and uptake of Zn and thus Zn efficiency, the lower Zn uptake by mycorrhized plants may be attributed to a decrease in the secretion of chelators. Root carbohydrates required for the synthesis of PS`s will in mycorrhizal plants partially be transported to microsymbiont, thus decreasing the potential for PS biosynthesis. In contrast, AM assisted the plants to take up more P provided that infected plants had an effective mycorrhizal infection.

In conclusion, neither the release of PS, the uptake of Zn, the retranslocation and/or remobilisation of Zn from older leaves to younger leaves nor AM could explain the difference in Zn efficiency as observed among the bread wheat cultivars in the field and in pot experiment. Differential resistance to low Zn availability in soils between durum wheat and other cereals species used in this thesis was attributed to the differences in the release of PS's. Among all cereals, the superior Zn efficiency of rye was likely linked with higher total uptake capacity for Zn. However, it is not clear whether this was due to uptake capacity or root morphology. This point should also be clarified in future studies.

Furthermore, the results obtained here indicated that although the various studied cerelas did not differ in any of mechanisms which were tested in plants grown in nutrient solution without rhizosphere processes, bread wheat and durum wheat cultivars grown in soil showed clear differences in Zn uptake, in such a way that, Zn-efficient bread wheat tended to take up more Zn from soil than Zn-inefficient bread and durum wheat cultivars. Up to a 4.7 fold higher Zn uptake relative to the total DTPA extractable Zn in the whole experimental, indicated an important role of rhizosphere processes which might have contributed to Zn mobilization but are not present in nutrient solutions. Therefore, future studies should focus their attention on experiments simulating rhizosphere processes related to the mobilization of Zn from soil.

11. Summary

Zinc (Zn) deficiency is one of the most widespread micronutritional disorder in crop plants, and occurs predominantly in calcareous soils of arid and semiarid regions. Zinc deficiency causes severe reductions in grain yield and also in nutritional quality of grains. To date, the mechanisms which are involved in Zn efficiency of cereals have not been clarified at a physiological level and mainly only two genotypes were used in such physiological studies. Therefore, it was the main goal of the present Ph.D. thesis to investigate the contribution of several different mechanisms that might contribute to Zn efficiency such as the release of Zn-mobilizing root exudates, inorganic Zn (Zn^{2+}) uptake by roots, Zn retranslocation or the symbiosis with arbuscular mycorrhiza (AM). For this purpose a large range of different cereal species was tested that showed differential Zn efficiencies in field studies conducted in Turkey.

To compare growth performances of various cereals, one rye, two bread wheat, and one durum wheat cultivars all differing in Zn efficiency were grown on a Zn-deficient soil under greenhouse conditions. Dry weight production under -Zn relative to +Zn decreased in the following order: rye cv. Aslim, bread wheat cv. Bezostaja, another bread wheat cv. BDME-10 and durum wheat cv. Kunduru-1149. This observation was in good accordance to the growth studies performed in the field.

To test the importance/contribution of AM in/to Zn nutrition of two bread wheat and one durum wheat cultivars, plants were grown on an extremely Zn-deficient soil in a pot experiment in the greenhouse. Irrespective of P supply and AM, all cultivars showed Zn uptake rates that reflected their Zn efficiency in the field and under greenhouse conditions. As

expected, mycorrhizal infection (%) enhanced the uptake of P in all cultivars, regardless of genotypical differences in Zn efficiency. However, under Zn deficiency AM led to lower Zn uptake. This decreased Zn uptake in the mycorrhizal plants coincided with a hindered growth pointing to an altered carbohydrate metabolism and/or partitioning in these plants. In general, no relationship could be found between Zn efficiency of wheat cultivars and the rate of mycorrhizal infection on the roots.

To elucidate physiological mechanisms of Zn efficiency, PS release of one rye, one triticale, seven bread wheat, and two durum wheat genotypes that markedly differ in Zn efficiency were investigated in nutrient solution culture. The results obtained in both experiments showed that Zn deficiency stimulated PS release in wheat and the low sensitivity of bread wheat cultivars compared to durum wheat cultivars to Zn deficiency coincided with high PS release supporting a role of PS in Zn efficiency. Comparing rye, triticale, and bread wheat cultivars, however, PS release did not correlate with the observed Zn efficiency in the field at low Zn availability, indicating that PS release cannot be regarded as a major physiological mechanism involved in Zn efficiency in cereals.

To test the role of the Zn uptake capacity for Zn efficiency, one rye, three bread wheat, and one durum wheat cultivars were grown in nutrient solution and short-term uptake of ^{65}Zn supplied as $ZnSO_4$ was determined in plants precultured under -/+ Zn. While Zn-efficient rye cv. Aslim showed the highest uptake rate, Zn-inefficient durum wheat cv. Kunduru-1149 had the lowest, suggesting that a low Zn uptake capacity of durum wheat might be an important reason for its higher susceptibility to Zn deficiency. However, the differential Zn efficiency among the bread wheat cultivars was not related to their capacity for the uptake of inorganic Zn.

Finally, the role of Zn retranslocation for the internal utilization efficiency of Zn was investigated in bread and durum wheat cultivars. There were no differences among these cultivars, neither in the translocation of foliar applied ^{65}Zn nor in retranslocation of pre-loaded ^{65}Zn from older leaves to younger tissues. Thus, no indication was found for an important role of Zn retranslocation for the expression of Zn efficiency in wheat.

All of the mechanisms which were tested in this Ph.D. thesis or in other studies cannot sufficiently explain the reason for the genotypical differences in Zn efficiency as observed in bread wheat. Although differential Zn efficiency in bread wheat as observed in field studies could be reproduced in pot experiments, all experiments that aimed to analyze individual components in Zn efficiency were unsuccessful to obtain significant differences among the bread wheat cultivars. Since these experiments were all conducted in nutrient solution, it is likely that a physiological mechanism that depends on the integrity of the rhizosphere triggers Zn efficiency. It is therefore suggested that future studies should focus on experiments that allow a reconstruction of rhizosphere processes as they are likely to occur in agricultural and natural soils.

12. Zusammenfassung

Zinkmangel (Zn) ist eine der verbreitetsten Mikronährstoffstörungen in Pflanzen, und tritt hauptsächlich auf kalkhaltigen Böden in ariden und semiariden Regionen auf. Zinkmangel führt zu erheblichen Kornertragseinbußen und beeinträchtig auch die Kornqualität hinsichtlich ihres Wertes für die menschliche Ernährung. Die Mechanismen, die eine Rolle für die Zn-Effizienz auf physiologischer Ebene bei Getreide spielen, sind bis heute nicht vollständig geklärt und meist wurden nur zwei verschiedene Genotypen verwendet. Das Ziel der vorliegenden Arbeit war es, den Beitrag, den verschiedene Mechanismen zur Zn-Effizienz leisten, zu untersuchen, wie z.B. die Abgabe von Zn-mobilisierenden Wurzelexsudaten, die Aufnahme von anorganischem Zn (Zn^{2+}) durch die Wurzeln, die Zn-Retranslokation innerhalb der Pflanze oder die Symbiose mit vesikulär-arbuskulären Mykorrhiza (VAM). Diese genannten Mechanismen wurden an einer großen Auswahl verschiedener Getreidearten, die unterschiedliche Zn-Effizienz in Feldstudien in der Türkei gezeigt haben, untersucht.

Um das Wachstum verschiedener Getreidearten zu untersuchen, wurden eine Roggensorte, zwei Brotweizensorten und eine Durumweizensorte auf einem Zn-Mangelboden in einem Gewächshaus angezogen. Die Trockengewichtsproduktion bezogen auf -Zn relativ zu +Zn nahm in folgender Reihenfolge ab: Róggensorte Aslim, Brotweizensorte Bezostaja, Brotweizensorte BDME-10 und Durumweizensorte Kunduru-1149. Diese Beobachtungen stimmten gut mit den Ergebnissen von Feldstudien überein.

Um den Beitrag von VAM zur Zn-Ernährung von zwei verschieden Brotweizensorten und einer Durumweizensorte zu untersuchen, wurden die Pflanzen in Gefäßen gefüllt mit einem extremem Zn-Mangelboden in einem Gewächshaus kultiviert. Unabhängig von der P-Versorgung und der Mykorrhizierung, widerspiegelte die Zn-Aufnahmerate aller Sorten ihrer im Feld und ihrer bei im Gewächshaus durchgeführten Gefäßversuche gezeigten Zn-Effizienz. Wie erwartet, erhöhte eine Mykorrhizierung der Pflanzen die P-Aufnahme aller Sorten unabhängig von genotypischen Unterschieden in der Zn-Effizienz. Allerdings verringerte die Mykorrhizierung die Zn-Aufnahme der Pflanzen unter Zn-Mangelbedingungen. Diese verminderte Zn-Aufnahme der mykorrhizierten Pflanzen deckte sich mit einem gehemmten Wachstum und deutet auf einen veränderten Kohlenhydratmetabolismus in diesen Pflanzen an. Im Algemeinen bestand keine Beziehung zwischen der Zn-Effizienz der Weizensorte und der Mykorrhizierungsrate ihrer Wurzeln.

Um die physiologischen Mechanismen der Zn-Effizienz näher zu beleuchten, wurde die Abgabe von Phytosiderophoren (PS) bei Roggen, Tritikale, sieben Brotweizensorten und zwei Durumweizensorten in Nährlösungskultur untersucht, wobei die verschiedenen Getreidesorten sich wesentlich in ihrer Zn-Effizienz unterschieden. Zn-Mangel stimulierte die PS-Abgabe bei Weizen. Die niedrige Empfindlichkeit gegenüber Zn-Mangel von Brotweizensorten im Vergleich zu Durumweizensorten stimmte mit einer hohen PS-Abgabe überein und unterstützt somit die Rolle von PS bei der Zn-Effizienz. Ein Vergleich von Roggen, Tritikale und Brotweizensorten zeigte allerdings keinen Zusammenhang zwischen PS-Abgabe und Zn-Angebot im Boden und somit kann die PS-Abgabe nicht als ein bedeutender physiologischer Mechanismus für die Zn-Effizienz in Getreide angesehen werden.

Um den Einfluß der Zn-Aufnahmekapazität auf die Zn-Effizienz von Weizenarten zu untersuchen, wurden an Roggen, drei Brotweizensorten und Durumweizen Kurzeitaufnahmestudien mit ^{65}Zn an Pflanzen durchgeführt, die auf -/+Zn in Nährlösung vorkultiviert wurden. Während die Zn-effiziente Roggensorte Aslim die höchste Aufnahmerate zeigte, hatte die Zn-ineffiziente Durumweizensorte Kunduru-1149 die Geringste. Dies läßt vermuten dass die niedrige Zn-Aufnahmekapazität von Durumweizen, eine große Rolle für seine hohe Empfindlichkeit gegenüber Zn-Mangel spielt. Allerdings konnte die unterschiedliche Zn-Effizienz verschiedener Brotweizensorten nicht mit ihrer Zn-Aufnahmekapazität in Beziehung gesetzt werden.

Schließlich wurde die Bedeutung, von der Zn-Retranslokation für die interne Zn-Nutzungseffizienz an Brot- und Durumweizensorten untersucht. Die verschiedenen Sorten unterschieden sich weder in der Translokation von Blatt-appliziertem ^{65}Zn noch in der Retranslokation von zuvor beladenem ^{65}Zn aus alten Blättern in junges Gewebe. Demzufolge gab es keinen Hinweis auf eine wichtige Rolle der Zn-Retranslokation für die Ausprägung von Zn-Effizienz bei Weizen.

Alle Mechanismen, die in der vorliegenden Arbeit oder auch in anderen Arbeiten untersucht wurden, können die genotypischen Unterschiede der Zn-Effizienz von verschieden Brotweizensorten nicht erklären. Obwohl die auf dem Feld beobachtete Zn-Effizienz von verschiedenen Brotweizensorten, auch in Gefäßversuchen reproduziert werden konnte, Einzelkomponenten der Zn-Effizienz keine signifikanten Unterschiede. Da es sich hierbei um Nährlösungsversuche handelte, ist es möglich, dass die physiologischen Mechanismen, die die Zn-Effizienz ausmachen, von der Unversehrtheit der Rhizospäre abhängen. Zukünftige Studien sollten auf Versuche konzentrieren, die die Rekonstruktion von Rhizosphärenprozesse erlauben, die höchstwahrscheinlich in landwirtschaftlichen und natürlichen Böden ablaufen.

13. References

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2) **Erenoğlu 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* 146, 241-250.

3) **Erenoğlu B**, Römheld V & Cakmak I (2001): Retranslocation of zinc from older leaves to younger leaves and roots in wheat cultivars differing in zinc efficiency. *In,* Plant nutrition- Food security and sustainability of agro-ecosystems. Eds. W J Horst et al. pp. 224-225. Kluwer Academic Publishers, Dordrecht, the Netherlands.

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