CHARACTERIZATION OF ZINC UPTAKE, TRANSLOCATION AND REMOBILIZATION IN HYDROPONICALLY GROWN *TRITICUM TURGIDUM* L. subsp. *DURUM* DESF. AND *TRITICUM TURGIDUM* L. subsp. *DICOCCOIDES* GENOTYPES

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GAMZE ALTINTAŞ

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APPROVED BY:

Prof. Dr. İsmail Çakmak (Dissertation Supervisor).....

Prof. Dr. Zehra Sayers.....

Asst. Prof. Alpay Taralp.....

Assoc. Prof. Batu Erman.....

Asst. Prof. Devrim Gözüaçık.....

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ABSTRACT

Zinc (Zn) is an essential trace element for all organisms. Cereal-based diets typically do not provide an adequate source for Zn nutrition of human beings, particularly when cereals are cultivated on Zn-deficient soils. This study investigates the potential of wild emmer wheat (*Triticum turgidum* L. subsp. *dicoccoides*) for better Zn-uptake, translocation and mobilization (retranslocation). These traits can be further utilized in breeding new genotypes with enriched grain Zn concentration. Solution culture experiments were conducted with selected wild emmer wheats (TTD 172, 24-39, TD 153, TD 531, TD 678, TTD 96, TTD 21, TTD 27, TD 536, TD 510) along with cultivated modern wheats (*Triticum turgidum* L. subsp. *durum* genotypes, Sarıçanak 98 and Balcalı 2000) for a comparison of findings. Genotypes tested at early growth stage showed large differences in root Zn uptake and in mobilization (retranslocation) from older leaves into roots and young parts of shoots. The differences found in root uptake and leaf mobilization of Zn among the genotypes were not related to the differences in seed concentrations of those genotypes used in the experiments. These results indicate that genotypic variation in seed Zn concentrations among and within the modern and wild tetraploid wheat genotypes seem to be not related to the differences in root Zn uptake rate or Zn mobilization rate from older leaves during the early growth stage under given conditions. It is concluded that for better understanding and characterization of genotypic variation in differential accumulation of Zn in seeds, an increasing attention should be paid to the i) mobilization ii) phloem transport and iii) seed deposition of Zn during late (generative) growth stage of plants.

Keywords: Zn, Wild emmer, durum wheat, dicoccoides, Zn-65

ÖZET

Çinko bütün organizmalar için eser miktarda gerekli olan bir elementtir. Tahıla dayalı beslenme şekilleri, özellikle bu tahıllar Zn bakımından eksik topraklarda yetiştirilmişse, insan sağlığı için gerekli olan yeterli düzeyde Zn'yi genellikle sağlayamamaktadır. Bu çalışma yabani makarnalık buğdayın (*Triticum turgidum* L. subsp. *dicoccoides*) daha iyi Zn-alınım, taşınım ve tekrar taşınım konusundaki potansiyelini araştırmak için yapılmıştır. Bu özellikler ileride tane Zn konsantrasyonu bakımından zengin yeni buğday türlerinin oluşturulmasında kullanılabilir. Bu çalışmada yabani makarnalık buğday genotipleri TTD 172, 24-39, TD 153, TD 531, TD 678, TTD 96, TTD 21, TTD 27, TD 536 ve TD 510 kullanılmış olup bulguları karşılaştırmak için de modern makarnalık buğday (*Triticum turgidum* L. subsp. *durum)* çeşitleri olan Sarıçanak 98 and Balcalı 2000 ile su kültürü denemeleri yapılmıştır. Erken büyüme evresinde test edilen genotipler kök Zn alınımı ve yaşlı yapraklardan köke ve yeşil aksamdaki genç dokulara Zn taşınımı için büyük farklılıklar göstermiştir. Zn'nin kökten alınımı ve yapraktan tekrar taşınımı için genotipler arasında bulunan farklar deneylerde kullanılan genotiplerin tohumlarındaki Zn konsantrasyonları ile ilişkili değildir. Bu sonuçlar gösteriyor ki verilen koşullarda ve erken büyüme safhasında tohumdaki Zn konsantrasyonun modern ve yabani tetraploid buğday genotipleri arasındaki genotipik varyasyonu kök Zn alınımı ve Zn'nun yaşlı yapraklardan tekrar taşınım oranına bağlı gözükmemektedir. Özet olarak, tanede Zn birikiminde görülen genotipik farkların daha iyi anlaşılması ve karakterize edilmesi için gerekli önem i) tekrar taşınıma, ii) floemde taşınıma ve iii) Zn'nun geç (jeneratif) büyümü safhasında tanede birkimine verilmelidir.

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Anahtar Kelimeler: Zn, yabani makarnalık buğday, durum buğdayı, dicoccoides, Zn-65

To my family and my funny valentine with all my heart...

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ABBREVIATIONS

C-O: Carbon oxygen bond

CPM: Counts per minute

DM : Dry matter or dry mass

DW: Dry weight

E. coli*: Escherichia coli*

G: Genotype

GXT: Genotype versus treatment

HMA: The Heavy Metal-ATPase

LSD: Least significant difference

MTP: Microsomal triglyceride transfer protein

NA: Nicotianamine

P-O: Phosphorus oxygen bond

PS: Phytosiderophore

T: Treatment

TD: *Triticum dicoccoides*

TTD: *Triticum turgidum dicoccoides*

WHO: World Health Organization

YSL: Yellow Stripe Like family of transporters

ZIP: Zinc/Iron Permease

Znt: Zinc Transporter gene

Zn-65: radioactive isotope of Zn

3D : Three dimensional

-Zn: Zn deficient treatment (10^{-8} M)

+Zn : Zn sufficient treatment (10^{-6} M Zn)

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1. INTRODUCTION

There are 14 essential minerals for optimum growth and development of plants and 12 of them are utilized by humans (Grusak and DellaPenna, 1999). Zinc is an essential micronutrient that is required for all known organisms. It is involved in catalytic processes of more than 300 enzymes and known to play significant role in gene expression, cell development and replication (Hambidge, 2000). Zinc also works with transcriptional regulatory proteins to stabilize them (Fox and Guerinot, 1998).

The UN report in 2004 underlines that micronutrient undernourishment affects more than half of the population on the world and the risk groups are preschool children, women at reproductive age and elderly people (Diaz, et al., 2003). Zinc deficiency is responsible for 800 000 child deaths per year (Micronutrient Initiative, 2006). Severe Zn deficiency symptoms are generally observed in rural and urban populations with low-income due to high consumption of the plant food based diets. The most affected regions on the world are Africa, Asia, and Latin America where the low-income limits the diversification of diet. In addition to dietary diversification, malnutrition due to Zn deficiency can be overcome by fortification of foods, supplementation with pharmaceutical products and biofortification of food crops with Zn. Biofortification is the process of generating micronutrient-rich crop varieties by conventional breeding methods, and it is where advances in technology meets with agricultural research to improve the food security and to enhance the quality of life.

The generation of biofortified crop genotypes requires the identification of efficient genotypes with enhanced micronutrient contents, the optimization of this genotypes for higher yield and for better tolerance to the environmental factors, so that new varieties deployed to farmers would be adopted (Ortiz-Monasterio, et al., 2007).

Although biofortification of crops with Zn requires the reveal of the underlying mechanisms of Zn uptake and translocation, it is still uncertain whether ion channels or divalent cation carrier are the predominant element of Zn uptake and the link between uptake and metabolic energy transduction has not been shown yet (Kochian, 1993).

The focus on genetic research for increased grain Zn accumulation would yield valuable outcomes and greatly contribute to conventional breeding efforts. However, there is still much to be understood about the physiological mechanisms involved in Zn accumulation, remobilization, partitioning and senescence. These mechanisms are often required to be studied in combination due to the fact that there is still no consensus on which mechanism is of greater importance for higher grain deposition. Nutrient solution culture is a convenient medium to study possible differences in uptake, transport and mobilization of Zn among different plant species and genotypes of the *Triticum* family. However, solution culture omits the interaction between root and soil and findings of hydroponic experiments often need to be confirmed by pot and field experiments.

The aim of this study is to evaluate the potential of wild emmer as a genetic source in biofortification of cultivated wheat with by elucidating the responses of selected wild emmer wheats to Zn uptake, translocation and along with commercial durum wheat cultivars under low or adequate Zn supply. Zn uptake, translocation and mobilization of 10 wild *Triticum diccocoides* and 2 modern wheat cultivars were investigated. Radiolabeled Zn (i.e. $^{65}ZnCl_2$) was employed to measure nmol concentrations of Zn in uptake and mobilization experiments by gamma counting. Additionally, Zn status of plants was determined by total Zn analysis by ICP-OES following acid digestion.

2. OVERVIEW

2.1 Zinc as an essential transition metal

Zinc is one of the most important micronutrients required for both plants and human beings (Marschner, 1995; Alloway, 2001). In contrast to many other physiologically important metals like iron and copper, Zn is colorless and diamagnetic transition metal which makes it difficult to detect and trace with simple spectroscopic methods. In addition, although Zn is involved in catalytic processes of more than 300 enzymes and more than 200 3D structures of proteins interacting with zinc are resolved (Andreini et al., 2008), the wide distribution of Zn among diverse proteins and enzymes causes a decline in Zn concentration and the dilute concentration of Zn makes it much more difficult to study (Maret, 2001).

2.1.1 Physical and chemical properties of zinc

In enzymes, Zn is one of the most abundant metal ions after Mg (Andreini et al., 2008). It is a group II transition metal and found as a highly stable and redox-inert ion at +2 oxidation state. With a radius of 0.74 Å and electrostatic affinity to negatively charged species, Zn plays role in many active centers of various enzymes having negatively charged residues.

Due to its higher electron affinity and strong Lewis acid character, it helps to create hydroxide ions for substrate attack. In addition to its role in generating the nucleophile, Zn polarizes P-O and C-O bonds of substrate and enhances the reaction by making substrates more electrophilic (Vallee and Auld, 1990).

The versatile coordination chemistry of Zn enables it to perform substrate binding and change in coordination geometry and number. The active centers containing Zn are generally accompanied by immobile N and O donors and mobile S donors which work in accordance with Zn's coordination numbers between 4 and 6 (Benini et al., 2004). Filled 3d shell of Zn results in kinetically labile coordination sphere which increases the turnover rates of Zn containing enzymes.

2.1.2 Biochemical properties of zinc

The steochemistry of Zn enables it to bind many proteins and enzymes and the three primary Zn-binding sites are structural, catalyic and cocatalytic (Auld, 2001); the enzyme examples for these sites are alcohol dehydrogenases, carbonic anhydrases and superoxide dismutases, respectively. The binding properties of Zn differ from site to site. For structural functions, Zn generally prefers four ligands which is generally a cysteine amino acid. For catalytic sites, a water molecule and a histidine are required and the other sites are occupied by any other S, N or O donors. In the cocatalytic sites for Zn, except from Cys, both histidine (His) accomponied by a water molecule and aspartic acid (Asp) or glutamic acid (Glu) can be found (Maret, 2005).

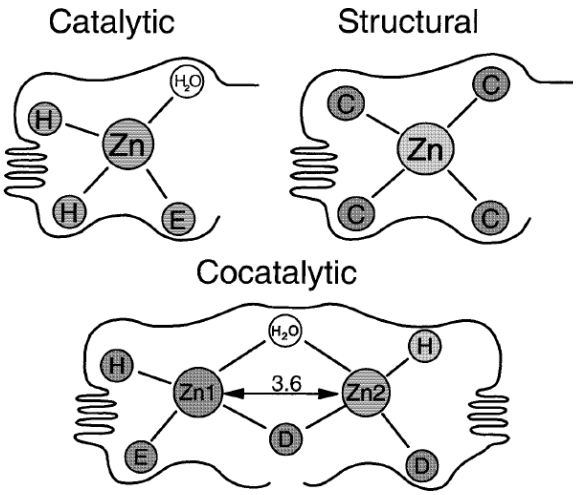

Fig. 2.1: Zinc binding sites in enzymes (Auld, 2001): catalytic (thermolysin (Matthews, 1988)), structural (alcohol dehydrogenase (Eklund & Branden, 1987)), cocatalytic (Aeromonas proteolytica aminopeptidase (Chevrier et al. 1994)). The letters C, D, E and H refers to the aminoacids, cysteine, aspartic acid, glutamic acid and histidine, respectively.

2.1.3 Proteins interacting with zinc

According to nomenclature of International Union of Biochemistry and Molecular Biology, the enzyme families are grouped into six classes: oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases and all six enzyme families have Zn binding members.

Carbonic anhydrase is discovered in 1940 as a first enzyme with Zn binding capability (Keilin et al.,1940) and the discovery of Zn enzymes continued with carboxypeptidase in 1954 (Vallee & Neurath, 1954). Due to its unique biochemical properties, Zn presents in the center of many enzymes' active sites. One of the largest group of Zn enzymes are Zn proteases such as endopeptidase thermolysin (Matthews, 1988), interstitial collagenase matrixin (Springman et al., 1990) and neurotoxins from Clostridium tetani and Clostridium botulinum (Giampietro & Montecucco, 1995). Zinc

cations are also found in binuclear form at the active centers of many Zn aminopeptidases like Methionyl aminopeptidase of E. coli. (Wilcox, 1996).

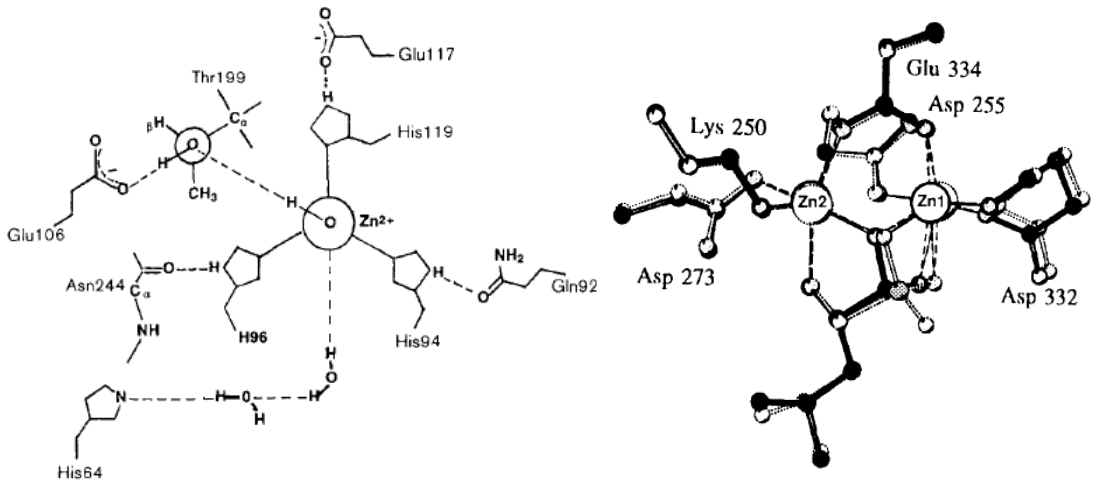

Figure 2.2 : Zinc at active sites of carbonic anhydrase and aminopeptidasea) a) Amino acid residues and hydrogen bonds at the active center of human CA II (Coleman, 1967). b) The binuclear Zn complex at the active center of leucyl aminopeptidase (Sträter et al., 1995).

The binding site for Zn is not limited with enzymes, DNA/RNA binding proteins, membrane lipids are the preferred binding sites of Zn. Zinc finger domain containing proteins which function as the regulatory proteins of transcription, site-specific modifications and chromatin structure are the largest class of Zn-binding proteins (Klug, 1999).

2.2 Zinc in human health

Zinc is an essential micronutrient for human health and well being. The first Zn related deficiency syndrome was published by Prasad et al. in 1961. In their publication, adolescent nutritional dwarfism that had been observed in mid-eastern countries was

associated with malnutrition in diet and Zn was pronounced as the major etiological factor of that syndrome. After being pronounced as an etiological factor, Zn was recognized as an important micronutrient for human nutrition.

In 1973, Barnes and Moynahan discovered an autosomal recessively inherited disease acrodermatitis enteropathica which is a rare disease with ZIP4 transporter defect in intestinal cells (Wang et al., 2002) and the patients cannot absorb Zn from their daily diets. After the discovery of Zn deficiency syndrome and disorders, in 1974, the Food and Nutrition Board of the US National Academy of Sciences declared Zn as an essential nutrient however more than three decades later of declaration over 30% of world population is suffering from zinc deficiency particularly in developing countries whose diets mainly constituted by cereal based food and soils with low zinc availability such as Turkey (Cakmak et al, 1999a), India, and Australia (Alloway, 2009). According to WHO, infants, young children and pregnant women are the predominant risk groups for Zn deficiency (WHO, 2006).

2.2.1 Zinc deficiency

One of the widespread micronutrient deficiencies in soil is Zn deficiency. The soil having insufficient plant available Zn for the optimum growth of plants is named as Zn deficient-soil. It is shown that 30% of cultivated soils on world and 50% of cultivated soils in Turkey and India are Zn-deficient (Sillanpää, 1990; Cakmak et al., 1996). The widespread problem with Zn deficiency in soils has been also reported in China and Western Australia. Calcareous soils with high pH, sandy soils and soils fertilized with high-phosphorous containing fertilizers are susceptible to Zn deficiency (Marschner, 1995). Although rye and pea species are considered more tolerant to Zn deficiency in soils, wheat, rice, maize are vulnerable to Zn deficiency (Chapman, 1966). The critical level of DTPA extractable Zn for wheat is found 0.75 mg kg^{-1} and corresponding level separates Zn deficient soils form non-deficient soils (Bansal et al., 1990).

Micronutrient deficiency is common among 40% of world's population (Graham and Welch, 1996). Zinc deficiency, one of the important micronutrient deficiencies, is also widespread in human populations especially in developing countries due to the high consumption of cereal-based products. In addition to low levels of Zn in cereals; especially for ones cultivated in Zn deficient soils, the amounts of phytic acid is very high in cereals. Phytic acid is a compound which reduces bioavailability of Zn (Hambidge, 2000).

In humans, the deficiency of Zn causes malabsorption syndrome, growth retardation, loss of appetite, immune dysfunction and infections on systemic level (Prasad, 1993). However in particular skin lesions, decreased wound healing, chronic liver disease, chronic renal disease and acrodermatitis are associated with Zn deficiency (Barnes & Moynahan, 1973). Acrodermatitis is a severe disease that can be lethal in the absence of treatment and related symptoms are alopecia, diarrhea, weight loss, reduced immune function, and neuropsychological instability (Aggett, 1983).

Decreased nerve conduction, neurophysiciatric disorders, mental lethargy and neurosensory disorders are the neurobiological outcomes of Zn deficiency. Infertility, retarded genital development, hypogonadism, thymic athropy are the other symptoms caused by inadequate Zn levels in human body (Prasad, 1993).

As a 2B element on periodic table, the counterparts of Zn are Cd and Hg. The toxicity of Cd and Hg are mainly due to their potential for displacement of Zn from its binding sites. Therefore, the deficiency of Zn is highly correlated with vulnerability to toxicity and carcinogenicity of its counterparts. The study by Costello and Franklin (Costello and Franklin, 1998) showed a lower prostate cancer development rate in men with moderate to high intake of Zn than men with low Zn intake. The elevated risk for prostate cancer can be caused by the suppressed immunological response (Delafuante, 1991). Zinc also plays role in activation of p38 and potassium channels which trigger cell death (Truong-Tran et al., 2001).

2.2.2 Zinc toxicity

Although Zn is an essential micronutrient for humans, it should be noted that it is also a heavy metal which can be toxic in higher doses of intake. Recommended dietary allowance (RDA) for Zn is 11 mg/day for men, 8 mg/day for women, 2–3 mg/day for infants, 5–9 mg/day for children (Trumbo et al., 2001). The LD50 dose of Zn intake is determined as 27 g Zn/day (ATSDRDTEM, 2005). However, as emetic dose of Zn is 225–400 mg, intake of 27 g Zn per day is likely impossible (Brown et al., 1964).

There is one case that is reported about death due to Zn intake more than lethal dose. The woman who took 28 g of Zn in the form of Zn sulfate developed tachycardia, hyperglycemia and died in five days due to hemorrhagic pancreatitis and renal failure (Fox, 1989). A recent study by showed that excess Zn sufficiency causes imbalance in Zn/Cu ratios resulting in cardiac abnormalities (Sanstead, 1995).

The symptoms immediately observed after uptake of toxic amounts of intake are nausea, vomiting, abdominal pain, lethargy, anemia, and dizziness (Porea et al., 2000). In contrast to studies concluding that Zn behaves as a neuromodulator (Tekada, 2000), Choi et al. showed that Zn can also behave as a neurotoxin (Choi et al., 1988). Naturally, the blood-brain barrier prevents the accumulation of toxic Zn in brain, however there is also one report that 12 g of metallic Zn swallowed by a boy caused lethargy and focal neurological deficits 3 days after intake (Murphy, 1970).

2.2.3 Strategies to manage human zinc deficiency

Several strategies have been developed and used for improvement of Zn deficiency in humans. These strategies can be classified in two groups i) dietary-based and ii) plant-based strategies where the letter one will be covered in part 2.3.2.

In humans, Zn deficiency symptoms start to be observed when the plasma Zn levels decrease to the range of 12-16 mg/100 ml. For the treatment usually an oral (220 mg/day) or intravenous (80 mg/day) Zn administration helps to eliminate the deficiency symptoms. However, this type of medical intervention is only limited to acute symptoms (Jeejeebhoy, 2007).

For dietary interventions, oral Zn supplements and Zn enrichment on foods can be used. Addition of micronutrients in the chemical form can be used to help target populations at increased risk. However constant supplementation, distribution and delivery of these chemicals are required for successful results. Based on a study conducted by World Bank in 1994, the average cost of Zn supplementation as Zn sulfate is US$25.7 per kg and additional costs like monitoring and analysis of Zn status will increase the average cost per person (WB, 1994). The physical and chemical forms of the supplementation should be addressed properly, the dosage should be managed and the supplementation frequency, toxicity and interference with other nutrients for every target group should be investigated (Plum et al., 2010).

2.3 Zinc in plants

Plants require various nutrients for healthy growth and reproduction. Zinc is one of the essential micronutrients. For adequate growth, typical leaf Zn concentration of most crop species is should be more than 15-20 mg Zn kg^{-1} DW (Marschner,1995). Although Zn is required in small amounts, Zn is crucial for biochemical reactions like photosynthesis (Randal and Bouma, 1973), sucrose biosynthesis (Singh and Gangwar, 1974; Shrotri et al., 1980), heat tolerance (Graham and McDonald, 2001). Zinc is also particularly important for structural and functional integrity of biological membranes, detoxification of reactive oxygen species and function and stability of number of proteins. (Broadley et al., 2007)

As Zn play essential roles in metabolic processes, the deficiency of Zn in plants results in observable symptoms. The characteristics of Zn deficiency in plants are dieback (necrosis on root apex), mottle leaf (spatial heterogeneous or interveinal chlorosis), bronzing (reddish-brown shade development), rosetting (auxin deficiency-like responses), goblet leaves (inward leaf lamina curling), little leaf (leaf size reduction) (Broadley et al., 2007).

In the following sections the routing of Zn from root uptake to seed deposition is explained. The potential of wild emmer wheats for biofortification of cultivated wheats with Zn is also discussed in light of the current literature.

2.3.1 Zinc uptake, translocation and remobilization

The transport of Zn from soil to seed starts at the rhizosphere zone where roots interact with the soil components (i.e. air, water, dissolved minerals and organic matter). Despite the studies with Arabidopsis showing that *ZIP* family Zn transporters play role in Zn uptake from rhizosphere (Grotz et al., 1998) and Zn deficiency upregulates two transcription factors for *bZIP* family (Assuncao et al., 2010), the uptake of Zn from roots are not well understood. The chemical familiarity and common transport mechanims between Zn and Cd (Grant et al., 1998) enabled to show that Zn is the competitive inhibitor of Cd uptake by *ZIP* transporters (Pence et al., 2000) and there is a shared uptake mechanism between Zn, Cu, Mn, Cd (Ramesh et al., 2003). Therefore, *ZIP* family proteins play role in the transport of not only Zn but also other micronutrients as well as Cd.

Following uptake by roots, micronutrients are transferred to shoot system which is a rate limiting step for micronutrient translocation to seeds (Palmgren et al.,2008). It is shown that there is a physiological difference in root-to-shoot micronutrient tranfer mechanisms between high grain Cd and low grain Cd species (Hart et al., 2006). As Cd

resembles to Zn in transport and protein interactions and Zn and Cd are loaded onto the xylem by similar mechanisms, difference between physiological root-to-shoot transfer mechanisms for Zn-efficient and Zn-inefficient species are anticipated (Uraguchi et al., 2009). *FRD3*, *FPN1*, *HMA2*, *HMA4*, *HMA5*, and *MTP3* genes are found to participated in translocation of metal micronutrients into xylem or across the root-shoot junction (Durrett et al., 2007; Courbot et al., 2007; Andres-Colas et al., 2006; Papoyan and Kochian, 2004; Arrivault et al., 2006). The functions of P_{1B} ATPase a transition metal pump, was elucidated (Williams and Mills, 2005), it is shown that products of *HMA* genes of P_{1B} ATPase family pump Zn and Cd pericycle to xylem vessels and facilitate xylem loading (Wong & Cobbett, 2009). Hanikenne et al. corroborated this findings showing that Zn hyperaccumulator Arabidopsis halleri had multiple copies of *HMA4* gene and elevated expression due to the multiple copies of that gene elevates Zn translocation from root to shoot (Hanikenne, 2008). *MTP3* were proved to be implicated in root to shoot loading of Zn in Fe deficiency (Arrivault et al., 2006).

Transpirational tension is the driving force of micronutrient transport to leaves in shoot xylem. Transpiration in glumes of wheat enables micronutrients to be carried in seed covering tissues however the unloading of nutrients from xylem followed by leaf uptake is not well understood. Similar to root uptake of Zn, xylem unloading of micronutrients is associated with *ZIP* gene expressions (Wintz et al., 2003) esp. *COPT* genes (Pozo et al., 2010). Elevated expression of *Znt1* and *Znt5* genes are observed in hyperaccumulator *T. caerulescens*, compared to nonaccumulator *T. arvense* (Pence et al., 2000; Hammond et al., 2006). It is also shown that *Znt1* gene is highly expressed in Zn accumulating cells (Küpper & Kochian, 2010). Studies with *T. caerulescens* and *A. halleri* showed that *MTP1* genes of cation diffusion facilitator (*CDF*) family which are responsible for heavy metal tolerance in shoot tissues and Zn sequestering in vacuoles (Desbrosses-Fonrouge et al., 2005) are highly expressed in Zn accumulating shoots (Becher et al., 2004).

A single vascular bundle connects seed to maternal tissue and the bundle lasts at the seed covering tissues without direct connection to seeds (Thorne, 1985). The transfer of micronutrients from seed covering tissues into the seed requires movement

of micronutrients from xylem to phloem despite the presence of apoplastic space between maternal and filial tissues behaving as a physical obstacle and absence of transpirational tension. Both movement of Zn from older leaves to younger leaves or roots and from glumes into seed has been established and uneven distributed expression of *HMA*, *ZIP*, *MTP*, *Nramp*, *NAS*, and *YSL* genes are observed in laser capture microdissection studies on barley grain vascular bundle, aluerone, endosperm, and embryo parts (Tauris et al., 2009). The uneven distribution of gene expressions suggests the specific roles of different cell types in micronutrient transport into the seed (Waters & Sankaran, 2011). Genes those are responsible for zinc uptake, translocation and remobilization are summarized on Fig 2.3 (Waters & Sankaran, 2011).

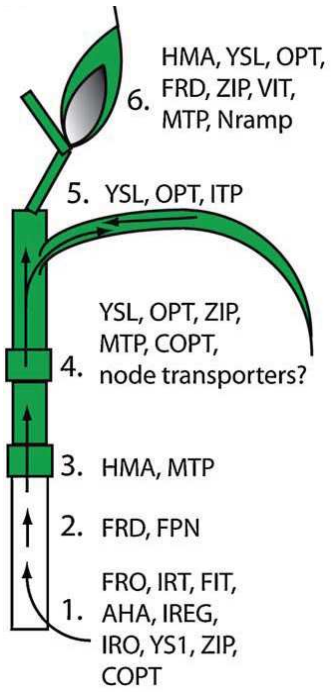

Fig. 2.3: Model of wheat plant showing the genes contributing in Zn translocation to the seed: 1, uptake from the rhizospere; 2, xylem loading; 3, root-to-shoot transfer; 4, distribution to the leaves or seed-covering tissues; 5, phloem loading for movement to seed; 6, loading into the seed. (Waters & Sankaran, 2011)

Phytosiderophores (PS) are organic compounds that are released into the rhizosphere and they form complexes with ferric iron (Fe^{3+}) in order to facilitate the uptake of Fe^{3+}. It is shown that PS can serve not only for Fe^{3+} but also for $Zn^{2+}$and Cu^{2+} (Treeby et al., 1989) and uptake of Zn^{2+} is also facilitated by PS (Wirén et al., 1996). PSs are not directly chelates Zn but the deficiency of Zn in the soil triggers iron

deficiency-induced phytosiderophore strategy to obtain Zn. Zinc is transported by this complex across root plasma membrane (Wiren et al., 1996).

Nicotianamine (NA) is a potential phloem chelator and binds Cu, Co, Fe (II), Fe (III), Mn, Ni and Zn (Higuchi et al., 1999). Nicotianamine is thought to play role in trafficing of metals these metals within the plant (Hell & Stephan et al., 1996). The overexpression of NA results in high Zn and Fe concentrations in developing seeds (Masuda et al., 2009). The study performed by Klatte et al. (2009) demonstrated that NA synthase mutants of Arabidopsis resulted in low concentrations of Fe in seeds and high concentration in leaves therefore NA is a critical micronutrient chelator playing role in micronutrient homeostasis by translocating micronutrients within vegetative fractions and by transporting them into seeds (Klatte et al., 2009).

The transport of micronutrient-NA complex by yellow-stripe like (YSL) proteins is revealed in maize and barley (Uena et al., 2009) and double mutant of these proteins are characterized to have decline in viability followed by decreased Fe, Zn and Cu concentration in seed showing that *YSL* proteins play role in micronutrient translocation between plant organs (Waters et al., 2006). In addition to *YSL* proteins, upregulated *OPT3* expression in Fe-deficiency studies on Arabidopsis suggests another inter-organ transport mechanism but the chelators and their ligands are not revealed yet (Wintz et al., 2003).

An existence of co-transport mechanism involving nitrogen and micronutrients are suspected after the observation that elevated N availability increased Zn translocation to wheat grain and thus seed Zn concentration while Zn availability remained unaffected (Kutman et al., 2010, Erenoğlu et al., 2011). Prior to this finding, Haydon and Cobbet (2007) have revealed that unspesific oligopeptides and certain amino acids play role in Zn transport as phloem chelators.

Nutrient remobilization is an important physiological process in senescence and grain filling. During senescence, which is the last state of leaf development, different types of nutrients (sugars, amino acids etc.) are transported to the grain. In wheat, 70-

80% of N and P and 40-50% of S in grain comes from senescing leaves by remobilization (Zhao et al., 1999). Rubisco (ribulose-1,5-biphosphate carboxylase/oxygenase) presents the major fraction of nitrogen in the chloroplasts where most of the organic nitrogen exists. Hence, at the beginning of senescence a decline in chloroplast stromal proteins takes place (Krupinska and Humbeck, 2004). Several hydrolytic enzymes are up regulated in senescing leaves to degrade leaf proteins to peptides and amino acids (Gepstein, 2004). It has been stated that expression levels of several classes of proteases (such as aspartic, serine, cysteine and metalloproteases) increased in senescing leaves (Fischer, 2010; Guo et al, 2004; Jukanti et al, 2008). *NAC* is shown to be one of the important genes in senescence, and *NAC* transcription factor accelerates senescence, enhances nutrient remobilization from leaves to developing grains and improves seed protein, Zn, Fe content in wheat (Uauy et al.,2006).

2.3.2 Biofortification strategies to increase zinc content of cereal grains

Approximately, a third of world's population suffers from Fe deficiency, in addition to 2 billion people suffering from Zn deficiency (Xiaoxi & Wu, 2007) and 1 billion people suffering from Se deficiency (Combs, 2001). The deficiencies are generally originated by consuming diets rich in staple foods but poor in fruits, vegatables, fish and animal products. However, increasing the consumption of nutrient rich products in daily diets, supplementation with nutrients and food fortification are not practical solutions to ameliorate the current deficiency status of world's whole population (Bouis, 2003; Timmer; 2003). In last decade, another solution for malnutrition is proposed (Graham & McDonald, 2001). The process of increasing the bioavailable content of essential nutrients in edible portions of cultivated crop species via agronomic interventions or genetic selection is called biofortification (White and Broadley, 2005). Biofortification is more amenable than traditional interventions due to the needless of uninterrupted investments, safe delivery systems, proper social infrastructure (White and Broadley, 2005). Fertilization, classical and molecular

breeding are the basic agricultural strategies to increase bioavailable nutrient content of crop species.

2.3.2.1 Zinc Fertilization

The availability of Zn in the soil for root uptake varies with soil moisture, soil pH, organic matter and $CaCO_3$ content of soil (Cakmak et al., 2010). Therefore, the bioavailability of Zn in soil affects directly root uptake and indirectly grain Zn concentration. Fertilization with Zn is a common and practical solution to correct soil-born Zn deficiency. There are a number of reports showing dramatic increases in yield as well as Zn concentration in the edible parts of crops by Zn fertilization (Peck et al., 2008; Rengel et al., 1999). Zinc fertilizers can be applied as foliar or soil application and it is shown that $ZnSO_4$ is a suitable form of Zn for fertiization and it effectively helps to increase grain Zn concentration in wheat (Yılmaz et al., 1997).

It is shown that foliar Zn applications are more powerful than soil applications in increasing grain Zn concentration. The combination of soil N or late Zn application with foliar Zn application was resulted in grain Zn concentration increase from 23 to 55 mg kg^{-1}, from12 to 29mg kg^{-1}, respectively (Cakmak et al., 2010). In addition to increased grain Zn concentration, reduced grain P and phytic acid concentration was also observed (Yılmaz et al., 1997). The reduction in antinutrients has also additive effect on bioavailability of Zn for humans.

As it is proposed that minimum 10 mg kg^{-1} Zn concentration increase in grain should be offered in order to have measurable biological impact (Pfeiffer and McClafferty, 2007), the effect of soil Zn applications combined with late foliar Zn fertilization could be a promising method for biofortification of grains with Zn (Cakmak et al., 2010). In genetic biofortification (plant breeding) attention is paid to Zn-rich wild

wheats to be exploited in breeding programs. *Triticum dicoccoides* is shown to be one of the highly promising wild wheat for improvements in grain Zn concentration (Cakmak et al., 2000).

2.3.2.2 Classical and molecular plant breeding

Classical and molecular plant breeding are powerful tools for increasing the grain Zn content of wheat. Screening of large germplasms for high seed zinc content constitutes the primary step of classical breeding studies. Selected genotypes are then crossed with high yielding, disease resistant and stress tolerant genotypes to ensure an optimum grain yield under contrasting regimes.

Phosphorus in seeds are stored as phytic acid (*myo*-inositol-1,2,3,4,5,6-hexa-*kis*-phosphate) and it constitutes 1-2% of seed dry weight. Phytic acid is known to having role in limiting bioavailabilty of Zn due to the its binding capacity to nutritionally important micronutrients such as calcium, iron and zinc (Sandstrom & Sandberg, 1992; Raboy, 2002). The study with volunteers having only low phytic acid containing maize in their diets showed enhanced Zn absorption (Adams, et al., 2002). The generation of new varities with grains containing “low phytate” may be useful to ameliorate human malnutrition (Raboy,2001). However recent studies pointed out that phytate is associated with enhanced seedling vigor and decreased aflotoxin activity and plays role in lower colon cancer rates (Grases et al., 2000; Morris, 1986). Thus, increasing grain Zn concentration rather than decreasing phytate activity seems to be more advantageous. In a bioavailability study using rats fed with wheat containing radioactivelly labeled Zn, it was shown that the negative effect of increased phytate content is not great enough to diminish the positive effects of increased grain Zn content (Welch, et al. 2005). Additionally, genotypes rich in Zn were supplied more bioavailable Zn to rats which again underlines that the biofortified genotypes may be the solution to overcome Zn deficiency (Welch, et al. 2005).

Another plant breeding approach to increase grain Zn would be to increase the grain protein content, because grain protein is suggested to be a sink for Zn (Persson et al., 2009). The increase in grain Zn and Fe concentrations with the help of N supply and synergetic effect of Zn and N supply on grain Zn concentration was demonstrated (Shi et al., 2010, Kutman et al., 2010). By N fertilization, enhancement in grain protein content can alleviate Zn status of grain by increasing the amount of available proteins in grains behaving as a sink for Zn deposition (Kutman et al., 2011).

According to Kutman et al. (2010), foliar applications of Zn and urea during grain-filling did not overcome N and Zn deficiency dependent yield loses. However, early publications demonstrated that foliar Zn and urea applications result in enhancement in grain Fe and Zn concentration (Varga and Svecnjak 2006, Yilmaz et al 1997). Soil or foliar Zn application accompanied by soil N fertilization was found to be very effective in increasing grain Zn concentration (Kutman et al., 2010). Therefore, wheat genotypes with higher grain protein content or genotypes responding N nutrition effectively should be adressed in breeding programs.

Breeding approaches are long term solutions for generating Zn-rich grains. However, the search for appropriate parents as a genetic source , crossing and back-crossing efforts, the persistence of new traits and the preference of new varieties over older ones are the restrictions for breeding approaches (Cakmak, 2008). Molecular plant breeding strategies are engaged with selecting desirable traits responsible for high grain Zn concentration using molecular biology tools and with various genetic modifications on present varieties by inserting those traits to increase seed Zn concentration. The genes that were described in section 2.3.1 and visualized on Figure 2.3 are the potential candidates for selection.

2.4 Wild emmer wheat (*Triticum diccocoides*) as a potential germplasm for high grain zinc

The most widely grown crops in the world are maize, wheat, rice and barley. Triticum (wheat) and Hordeum (barley) are the members of *Triticeae tribe*, the Poaceae subfamily of grass family. Among four crops, wheat cultivation ranks second after maize. *Triticum diccocoides*, the wild progenitor of wheat was the most important staple crop in Fertile crescent since early Neolithic sites until early Bronze Age and its importance remained till our age as a most important staple crop in Europe and West Asia.

The wheat is descended from small-grained grasses that are grown on Fertile Crescent in the Middle Asia. The first natural hybridization occurred between 10000 and 40000 years ago and the first ancestors of wheat are accepted as *Triticum urartu* which is wild einkorn wheat and a grass related to *Aegilops speltoides* which is a wild goat grass. However second ancestor became extinct. The hybridization of first ancestors created *Triticum diccocoides* which is known as wild emmer or emmer wheat. The wild emmer is the first cultivated wheat, however, another hybridization of *Triticum diccocoides* resulted in *Triticum durum* which is the modern durum wheat used in pasta making. The second hybridization of *Triticum diccocoides* occured with *Aegilops tauschii* created *Triticum aestivum* which later descended to the modern species of wheat used in bread making.

It has been reported that there exists genotypic differences in micronutrient use efficiency of crops which results from differences in uptake, transport and utilizations of nutrients (Rengel, 2001). Recently, the search for genetic traits of micronutrient-rich crops had yielded a potential gene. On chromosome 6B of T*riticum turgidum* ssp. *dicoccoides* was proven to be associated with grain protein (Joppa and Cantrell, 1990) and Zn and Fe concentrations (Cakmak et al.,2004). The gene *GpcB1* on 6BS is shown to regulate senescence and thus affect the concentration of Fe and Zn in the grain (Uauy et al., 2006).

Zn deficiency is one of the major problems threatening human population causing growth retardation, mental lathergy, immune dysfunction and infertility (Prasad, 1993). Although a diversified diet contains adequate levels of Zn for human health, the use of inherently low Zn containing cereals as a major food source especially in non-developed and developing countries causes the continuity of that major problem. Dietary-based and plant-based strategies are two solutions for managing Zn deficiency. The high cost and necessity for continuing effort in dietary-based interventions makes plant-based strategies more promising and more affordable. Fertilization, classical and modern breeding are the plant-based methods to solve Zn deficiency problem. However, as a short-term solution, fertilization is rapid but expensive and laborious. For long-term solutions, new varieties with enhanced nutrient content should be generated via classical or modern breeding methods. With the aim of generating new varieties with enhanced Zn bioavailability, genotypes having enough variation in seed Zn accumulation should be investigated. Although modern durum wheat genotypes were improved for better yield, they are poor genetic resources for breeding programs. Chatzav et al.(2010) found that seed Zn, Fe and protein concentrations in wild emmer genotypes were about two-fold greater than in the modern wheat genotypes. Study with 825 accessions of wild emmer wheat (*Triticum turgidum* L. subsp. *diccocoides*) showed variation from 14 to 190 mg Zn kg^{-1} in grain Zn concentration (Cakmak, et al. 2004). Wild emmer wheat genotypes having great potential for generating new enhanced varities and having enough variation in seed Zn content were selected to be used in this study.

The aim of this research is to characterize Zn uptake, transport and remobilization among hydroponically grown *Triticum turgidum* L. subsp. *durum* cultivars and wild emmer (*Triticum turgidum* L. subsp. *diccocoides*) genotypes and to elucidate the potential of wild emmer (*Triticum turgidum* L. subsp. *diccocoides*) genotypes over *Triticum turgidum* L. subsp. *durum* genotypes for biofortification by increase in grain Zn concentration. In order to understand the difference between wild emmer and modern durum wheat in Zn uptake from the growth medium, translocation to shoot, re-mobilization from old leaves and deposition of Zn into grains, two genotypes of *Triticum turgidum* L. subsp. *durum* and 10 genotypes of wild emmer (*Triticum turgidum* L. subsp. *diccocoides*) were used. ^{65}Zn was used for the monitor of Zn movements through solution to plant or through foliar application and the

mobilization and partitioning of Zn is investigated both chemically by ICP and radioactively by gamma counter.

3. MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Seed Material

Seed of 10 *Triticum turgidum* L. subsp. *dicoccoides* (i.e. TTD 172, 24-39, TD 153, TD 531, TD 678, TTD 96, TTD 21, TTD 27, TD 536, TD 510) and two *Triticum turgidum* L. subsp. *durum* (i.e. Sarıçanak 98, and Balcalı 2000) were initially obtained from Çukurova University Field Crops Department (Dr. Hakan Özkan) and then grown over 2 years under same conditions in field by Sabanci University to use in this study.

3.1.2 Chemicals

All chemicals were obtained from Riedel de Haen (Germany), Merck (Germany), Sigma (US) and Fluka (Switzerland). Radioactive Zn-65 source was

purchased from Polatom, Czech Republic in the form of $^{65}ZnCl_2$ with a specific activity of 20 MBq mg^{-1} Zn.

3.1.3 Nutrient Solutions

The compositions of nutrient solutions are explained in the following method sections.

3.1.4 Equipment

All equipment used in this research is listed in Appendix A.

3.2 METHODS

3.2.1 Zn-65 uptake and translocation experiment

Initially all seeds (*Triticum turgidum* L. subsp *dicoccoides* genotypes TTD172, 24-39, TD 153, TD 531, TD 678, TTD 96, TTD 21, TTD 27, TD 536, TD 510 and *Triticum turgidum* L. subsp. *durum* genotypes Sarıçanak 98, and Balcalı 2000) were sterilized in 80% ethanol for 2 min, rinsed with ddH_2O and placed on moistened filter

paper in a Petri dish. Following keeping for five days at 4°C, seeds were transferred into the perlite and germinated for five days. The germinated seedlings were selected for homogeneity and then transferred into pots containing 2.7 L of continuously aerated nutrient solution with the following composition. 2000 µM $Ca(NO_3)_2$, 1000 µM $MgSO_4$, 100 µM KCl, 200 µM KH_2PO_4, 700 µM K_2SO_4, 10 µM H_3BO_3, 0.5 µM $MnSO_4$, 0.2 µM $CuSO_4$, 0.01 µM $(NH_4)_6Mo_7O_{24}$, 100 µM FeEDTA. Low and adequate treatments of Zn received 0.05 and 1 µM $ZnSO_4$ respectively.

Plants were grown in a computer controlled growth chamber for 9 days (light intensity: 700 µmol m^{-2} s^{-1}, light/dark cycle: 16/8 hrs, temperature: 24/20^{o}C, humidity: 65-75%) and the nutrient solutions were refreshed every 3 days. On day 9, half of the plants grown under low or adequate Zn were supplied with 1 µM $ZnSO_4$ labeled with 77 KBq Zn-65. The other half of the plants was reserved for tissue Zn analysis by ICP-OES.

Following Zn-65 treatments, nutrient solutions were sampled at 15 min intervals to determine the decrease in activity of Zn-65 using a gamma counter (Perkin Emler 2480 WIZARD^2 Automatic Gamma Counter). On the third sampling (i.e. at 45 min) Zn-65 activity was estimated to be reduced by half and all solutions were quickly renewed with the non-radioactive version. All plants were harvested as shoot and root samples following 24 h after the initial Zn-65 treatment.

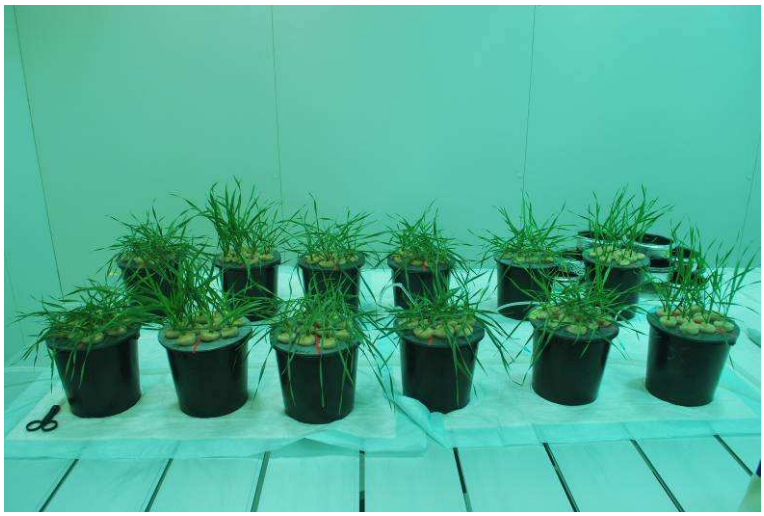

Fig. 3.1 Plants used for uptake experiment before Zn-65 treatment

The activity of Zn-65 in the root and shoot tissues were measured by a gamma counter. The data collected as counts per minute (CPM) were converted to Zn concentration using standards of known activity and concentration.

For the determination of Zn in shoots and roots, samples that are washed with distilled water just after harvest were dry ashed (550°C for 8 hours) and diluted in 5 % HNO_3 following by filtration through blue ribbon filters prior to measurement of Zn concentration by ICP-OES.

The dry mass of all harvested plant samples (i.e. shoots and roots) were determined after drying the samples in a forced oven at 65 °C until complete dryness.

In order to calculate the absorption and translocation rates of zinc, the data of absorbed zinc per root, shoot and total biomass (shoot+root) was used. All treatments had five replicates and the statistical analyses were done according to Student's t-test by JMP 5.0.1a statistical software. The average of all replicates and the interactions of genotype(G), treatment(T), genotype X treatment (GxT) and $LSD_{0.05}$ levels are evaluated.

3.2.2 Zn-65 retranslocation experiment

The remobilization (retranslocation) of zinc from old leaves to developing tissues was investigated by a nutrient solution experiment. All plants (*Triticum turgidum* L. subsp *dicoccoides* genotypes TTD172, 24-39, TD 153, TD 531, TD 678, TTD 96, TTD 21, TTD 27, TD 536, TD 510 and *Triticum turgidum* L. subsp. *durum*

genotypes Sarıçanak 98, and Balcalı 2000) were germinated and grown as indicated in section 3.2.1 with some modifications.

Different from the uptake experiment, seeds were vernalized for 22 days and the plants were harvested at 24 days after transfer to nutrient solution. For tracing the retranslocated portion of Zn from the oldest leaf to other plant parts, leaf tip of (approximately 4 cm) of the oldest leaf was treated with 0.2 % (w/w) $ZnSO_4$ solution containing 0.02 % Tween®20 labeled with 1480 KBq of Zn-65.

Each plant's oldest leaf tip was applied for 5 seconds for 3 times. Leaf application was repeated for 3 times in 8 h intervals. All plants were harvested 5 days after the first leaf application. At harvest plants were separated in three sections i) Zn-65 applied leaf tip, ii) reminder of shoot and iii) root, and all sections sampled were placed in to scintillation vials for Zn-65 activity measurements. Prior to activity measurements all leaf tips with Zn-65 applications were rinsed with ddH_20, 10 mM $CaCl_2$ and finally 2 % $ZnSO_4$ for 5 min to remove excess Zn adhered on the leaf surface and existing in leaf apoplast that is not taken up into the leaf tissue.

Fig. 3.2 Plants before Zn-65 foliar application (left) and Zn-65 foliar application (right).

Similar to the root Zn-65 uptake experiment, half of the plants were reserved for determination of Zn concentration and dry matter production in shoots and roots.

All treatments were performed in four replicates. The CPM (count per minute) data was used for statistical analysis performed by student's t-test of JMP 5.0.1a software. The Zn-65 activity in all samples were calculated and expressed in percent for all sections of application leaf, remainder of shoot and root parts and genotype (G), treatment (T) and genotype x treatment (GxT) $LSD_{0.05}$ levels were evaluated.

4. RESULTS

4.2.1 Zn-65 uptake and translocation experiment

As shown on Fig. 4.1, low (-Zn) and adequate (+Zn) Zn treated plants looked similar and healthy on the day of Zn-65 treatment (i.e. 9 days after transplant to nutrient solution). The low Zn plants had no apparent shoot Zn deficiency symptoms such as stunting, chlorosis or necrosis (Fig 4.1), but low Zn plants had less tissue Zn concentrations than the adequate Zn plants (see below).

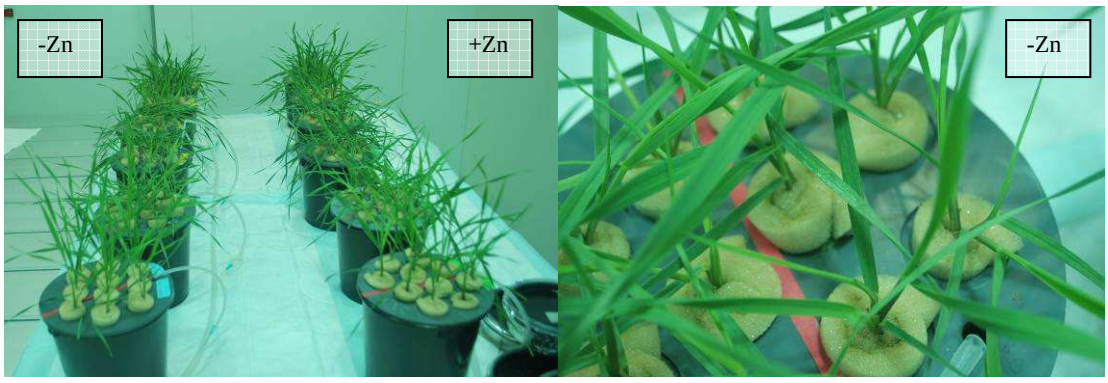

Fig. 4.1 Growth of low and adequate Zn plants on 9 days after transplant to nutrient solution.

Table 4.1 shows the effect of low (-Zn: 0.05 µM $ZnSO_4$) and adequate (+Zn: 1 µM $ZnSO_4$) Zn treatments on shoot and root dry matter production of the experimental plants at harvest. Compared to +Zn conditions (i.e. control treatment) shoot dry matter production was slightly reduced when plants were supplied with –Zn. Shoot dry matter in –Zn treatment ranged between 133 mg $plant^{-1}$ (Balcali 2000) and 344 mg $plant^{-1}$ (TTD 27) with a mean value of 210 mg $plant^{-1}$ whereas shoot dry matter in +Zn treatment ranged between 132 mg $plant^{-1}$ (Balcali 2000) and 352 mg $plant^{-1}$ (TTD 27) with a mean value of 227 mg $plant^{-1}$ (Table 4.1). At harvest all *T. dicoccoides* genotypes produced a remarkably higher shoot biomass compared to the cultivated *T. durum* Desf wheats. The Zn efficiency values calculated by the –Zn:+Zn biomass weight ratio ranged between 79 % (TD 510) and 109 % (TTD 96) with a mean value of 93 % (Table 4.1). In other words, plants treated with –Zn could produce, in average, 93 % of the shoot dry matter of plants treated with +Zn. In contrast to shoot dry matter production, root biomass was either not affected or slightly increased upon –Zn treatment. Average root dry weight was 170 mg $plant^{-1}$ for –Zn and 162 mg $plant^{-1}$ for +Zn plants. Consequently, the calculated Zn efficiency value for roots was 107 % in average (Table 4.1). In summary, *T. dicoccoides* and *T. durum* Desf genotypes generally responded to mild Zn deficiency stress by significantly reducing shoot and increasing root dry matter production. There was no evidence for a superior Zn efficiency of *T. dicoccoides* over *T. durum* genotypes with the exception of TTD 96. There was also no significant difference among the genotypes concerning the response to varied Zn supply, revealing that GxZn interaction was not significant and that all genotypes responded more or less similar upon Zn deprivation (Table 4.1).

Table 4.1 Effect of low (-Zn: 0.05 µM $ZnSO_4$) and adequate (+Zn: 1 µM $ZnSO_4$) Zn supply on shoot and root dry matter production of experimental plants (*Triticum turgidum* L. subsp. *durum* genotypes Sarıçanak 98, and Balcalı 2000 and *Triticum turgidum* L. subsp *dicoccoides* genotypes TTD172, 24-39, TD 153, TD 531, TD 678, TTD 96, TTD 21, TTD 27, TD 536, TD 510) at harvest on 10 days after transplant to nutrient solution. Zinc efficiency values were calculated by the ratio of dry matter production at –Zn to that of +Zn and expressed as percentage.

Genotype	Shoot			Root		
	-Zn	+Zn	Zn efficiency	-Zn	+Zn	Zn efficiency
	(mg plant^{-1})		(%)	(mg plant^{-1})		(%)
Saricanak 98	160	175	91	106	101	104
Balcali 2000	133	132	101	96	71	134
TTD 172	175	189	93	120	113	106
24-39	190	200	95	138	147	94
TD 153	225	234	96	210	169	124
TD 531	208	257	81	223	208	108
TD 678	235	240	98	155	145	107
TTD 96	227	208	109	200	183	109
TTD 21	171	198	86	176	155	114
TTD 27	344	352	98	246	258	96
TD 536	194	217	90	166	161	103
TD 510	259	327	79	210	236	89
Mean	210	227	93	170	162	107
$LSD_{0.05}$(G, Zn, GxZn)	(30, 12, NS)			(19, 8, NS)		

Shoot Zn concentration in –Zn treatment ranged between 11.7 mg kg^{-1} (Sarıçanak 98) and 24.8 mg kg^{-1} (TTD 21) with a mean value of 16.7 mg kg^{-1} whereas shoot Zn concentration in +Zn treatment ranged between 74.1 mg kg^{-1} (Sarıçanak 98) and 99.9 mg kg^{-1} (TTD 27) with a mean value of 82.4 mg kg^{-1} (Table 4.2). Among the wheat genotypes, *T. durum* Desf. Sarıçanak 98 had the lowest and and *T. dicoccoides* TTD 27 and TTD 21 had the highest shoot Zn concentrations irrespective of the Zn supply during plant growth. Concentration of Zn in roots ranged between 13.8 mg kg^{-1} (TD 510) and 27.3 mg kg^{-1} (TTD 172) in –Zn and 52.7 mg kg^{-1} (TD 678) and 145.6 mg kg^{-1} (Sarıçanak 98) in +Zn treatments mg kg^{-1}. The average root Zn concentration was 19.9 mg kg^{-1} and 76.2 mg kg^{-1} in –Zn and +Zn treatments respectively. The low and adequate treatments of Zn had resulted in about 4-fold difference in tissue Zn concentrations (Table 4.2) although the shoot Zn concentrations remained at around the marginal Zn deficiency level of 15-20 mg kg^{-1} (see Table 4.2) suggesting that the experimental plants had mild Zn deficiency at the time of harvest.

Zinc status of plants can be affected by the seed Zn reserve. This phenomenon is pronounced particularly in plants grown under limited Zn conditions. To evaluate the "seed reserve" phenomenon, seeds of all wheat genotypes used in the study were tested for total Zn concentration and the results are provided along with the shoot and root Zn concentrations (Table 4.2). Thus, seed Zn concentrations had generally no effect on shoot or root Zn concentrations (Table 4.2). As an example, genotypes with similar shoot and root Zn values had significantly different Zn concentrations in their seeds (e.g. TD 531 and TD 678) (Table 4.2). Finally, analysis of data revealed that genotype, treatment and the genotype by treatment interaction were statistically significant for both shoot and root data (Table 4.2). The variation in seed Zn was also statistically significant, although this was not translated to either shoot or root Zn results despite of the exceptionally high Zn in the seed of the *T. dicoccoides* cv. 24-39. In average, seeds of *T. dicoccoides* genotypes had about 1.8 fold higher Zn concentration in seeds compared to *T. durum* Desf. genotypes. Seed Zn also exhibited a broad variation in *T. dicoccoides* genotypes ranging between 131.3 mg kg^{-1} (24-39) and 45.4 mg kg^{-1} (TD 153) (Table 4.2).

Table 4.2 Effect of low (-Zn: 0.05 μM $ZnSO_4$) and adequate (+Zn: 1 μM $ZnSO_4$) Zn supply on shoot and root Zn concentration of experimental plants (*Triticum turgidum* L. subsp. *durum* genotypes Sarıçanak 98, and Balcalı 2000 and *Triticum turgidum* L. subsp *dicoccoides* genotypes TTD172, 24-39, TD 153, TD 531, TD 678, TTD 96, TTD 21, TTD 27, TD 536, TD 510) at harvest on 10 days after transplant to nutrient solution. The initial seed Zn concentrations are also provided to evaluate the possible effect of seed Zn to that of shoot and root Zn concentrations.

Genotype	Shoot		Root		Seed
	-Zn	+Zn	-Zn	+Zn	
			(mg kg^{-1} DW)		
Saricanak 98	11.7	74.1	16.4	145.6	39.7
Balcali 2000	13.6	90.4	22.2	114.6	35.2
TTD 172	14.2	85.1	27.3	67.5	59.4
24-39	19.5	91.0	21.4	64.6	131.3
TD 153	13.6	82.7	16.0	74.6	44.5
TD 531	14.7	74.6	23.2	62.7	47.2
TD 678	15.0	82.6	20.3	52.7	72.9
TTD 96	18.1	75.5	18.1	60.5	71.0
TTD 21	24.8	80.1	25.2	53.4	69.1
TTD 27	24.2	99.9	19.4	73.2	63.4
TD 536	16.0	74.9	15.5	74.6	49.0
TD 510	15.0	78.0	13.8	70.0	51.3
Mean	16.7	82.4	19.9	76.2	61.2
$LSD_{0.05}$(G, Zn, GxZn)	(7.0, 2.7, 10)		(7.5, 2.8, 10.5)		(1.4, - , -)

Root uptake, shoot transport and distribution within plants of ^{65}Zn were determined by short time application of Zn-65 to the growth medium. The technique enables measurement of nmol quantities of Zn in the harvested plant parts by gamma counting. The results were expressed both as per plant (Table 4.3) and unit root dry wt. (Table 4.4) for a complete evaluation of possible differences in Zn-65 uptake of the wheat genotypes tested.

Both *T. durum* Desf. and *T. dicoccoides* genotypes responded to –Zn treatment with induction of Zn uptake per plant (Table 4.3). There was an average of 6.5 fold increase in shoot Zn uptake and 2.2 fold increase in root Zn uptake per plant as a result of –Zn treatment. Consequently, genotypes expressed a large variation in shoot Zn

uptake per plant, particularly in –Zn treatment (Table 4.3). Among the *T. durum* Desf. genotypes, particularly Sarıçanak 98 expressed an induced Zn uptake to shoot, root and whole biomass in the –Zn treatment, although this induction remained much below the average of *T. dicoccoides* genotypes. Among the *T. dicoccoides* genotypes, an extreme case was of TD 531 in which Zn accumulation in shoot was induced up to 103.5 nmol plant^{-1} 24 h^{-1} in the low Zn treatment compared to 5.8 nmol plant^{-1} 24 h^{-1} of the control (Table 4.3). Similarly, TTD 27 and TTD 21 also induced Zn uptake per plant significantly higher than the other genotypes tested in the study (Table 4.3).

Table 4.3. Effect of low (-Zn: 0.05 μM $ZnSO_4$) and adequate (+Zn: 1 μM $ZnSO_4$) Zn supply on Zn uptake by shoot, root and whole biomass (i.e. shoot+root) of individual plants (*Triticum turgidum* L. subsp. *durum* genotypes Sarıçanak 98, and Balcalı 2000 and *Triticum turgidum* L. subsp *dicoccoides* genotypes TTD172, 24-39, TD 153, TD 531, TD 678, TTD 96, TTD 21, TTD 27, TD 536, TD 510). All plants were grown in Zn-65 labeled uptake solution for 45 min. Individual plants were harvested as shoot and root separately 24 h after the uptake period (i.e. 45 min) to achieve sufficient translocation rates for activity measurements in shoots.

Genotype	Shoot		Root		Whole biomass	
	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn
	(nmol plant^{-1} 24 h^{-1})		(nmol plant^{-1} 45 min^{-1})		(nmol plant^{-1} 45 min^{-1})	
Saricanak 98	33.7	8.7	17.6	11.9	51.3	20.7
Balcali 2000	14.8	5.9	13.5	10.4	28.3	16.3
TTD 172	15.5	6.3	13.2	5.7	28.7	12.0
24-39	14.7	5.3	11.8	6.2	26.6	11.5
TD 153	58.5	7.7	27.1	12.6	85.6	20.3
TD 531	103.5	5.8	24.7	10.3	128.2	16.1
TD 678	28.1	11.2	14.2	10.1	42.4	21.3
TTD 96	19.9	6.8	18.0	11.6	37.9	18.5
TTD 21	70.9	5.4	35.9	7.8	106.8	13.2
TTD 27	110.4	11.9	29.7	13.0	140.0	24.9
TD 536	40.0	3.1	26.4	6.0	66.3	9.1
TD 510	51.3	7.9	31.4	11.3	82.7	19.2
Mean	46.8	7.2	21.9	9.8	68.7	16.9
$LSD_{0.05}$(G, Zn, GxZn)	(5.5,2.2, 7.7)		(2.6 ,1.1, 3.6)		(7.2, 3.0, 10.2)	

Root uptake and shoot translocation rates are other important parameters for evaluation of the Zn uptake performance of wheat genotypes. Both root uptake and shoot translocation rate of Zn are calculated over one gram of dry root mass for a given

period of time (i.e. 45 min). Translocation efficiency is calculated by the shoot translocation to root uptake ratio and indicates the ability of a genotype to allocate Zn preferentially in the shoot rather than the root. The root uptake, shoot translocation and translocation efficiency values for all wheat genotypes are provided in Table 4.4. Root Zn uptake and shoot translocation rates were induced significantly in the –Zn treatment. Root Zn uptake rate varied between 184.1 nmol g^{-1} root DW 45 min^{-1} (24-39) and 605.9 nmol g^{-1} root DW 45 min^{-1} (TTD 21) with a mean value of 386.1 nmol g^{-1} root DW 45 min^{-1} in –Zn and 56.1 nmol g^{-1} root DW 45 min^{-1} (TD 536) and 228.5 nmol g^{-1} root DW 45 min^{-1} (Balcalı 2000) with a mean value of 115.1 nmol g^{-1} root DW 45 min^{-1} in the +Zn treatment (Table 4.4). Shoot Zn translocation rate varied between 100.3 nmol g^{-1} root DW 45 min^{-1} (24-39) and 464.2 nmol g^{-1} root DW 45 min^{-1} (TD 531) with a mean value of 256.0 nmol g^{-1} root DW 45 min^{-1} in –Zn and 19.0 nmol g^{-1} root DW 45 min^{-1} (TD 536) and 86.7 nmol g^{-1} root DW 45 min^{-1} (Sarıçanak 98) with a mean value of 48.4 nmol g^{-1} root DW 45 min^{-1} in +Zn treatment (Table 4.4). Results indicate a substantial variation in root uptake and shoot translocation of *T. dicoccoides* genotypes. There were also significant differences in root uptake and shoot translocation rates of *T. durum* Desf. genotypes and Sarıçanak 98 performed above the average of all genotypes (Table 4.4). Among the genotypes tested, cultivated wheats interestingly had higher root uptake and shoot translocation rates than their wild predecessors when supplied with sufficient Zn, whereas in –Zn conditions cultivated wheats ranked 4^{th} (Sarıçanak 98) and 8^{th} (Balcalı 2000) out of 12 genotypes. Under low Zn supply *T. dicoccoides* genotypes TTD 21, TD 531 and TTD 27 performed with the highest root uptake as well as highest shoot translocation rates. These *T. dicoccoides* genotypes also exhibited the highest translocation efficiencies compared to other genotypes. Translocation efficiencies of Sarıçanak 98 and Balcalı 2000 ranked 5^{th} and 12^{th} respectively (Table 4.4).

Table 4.4. Effect of low (-Zn: 0.05 μM $ZnSO_4$) and adequate (+Zn: 1 μM $ZnSO_4$) Zn supply on Zn-65 root uptake, shoot translocation rate and root-to-shoot translocation efficiency of individual plants (*Triticum turgidum* L. subsp. *durum* genotypes Sarıçanak 98, and Balcalı 2000 and *Triticum turgidum* L. subsp *dicoccoides* genotypes TTD172, 24-39, TD 153, TD 531, TD 678, TTD 96, TTD 21, TTD 27, TD 536, TD 510).

Genotype	Root uptake rate		Shoot translocation rate		Translocation efficiency	
	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn
	(nmol g^{-1} root DW 45 min^{-1})		(nmol g^{-1} root DW 24 h^{-1})		(%)	
Saricanak 98	496	205	328	87	66	42
Balcali 2000	295	229	154	82	52	36
TTD 172	240	105	129	55	54	52
24-39	184	79	100	36	53	46
TD 153	408	120	279	46	68	37
TD 531	575	78	464	28	81	36
TD 678	274	147	181	78	65	53
TTD 96	192	101	101	37	53	37
TTD 21	606	86	402	35	66	41
TTD 27	569	96	449	46	79	48
TD 536	398	56	239	19	60	34
TD 510	398	81	247	33	62	41
Mean	386	115	256	48	63	42
$LSD_{0.05}$(G, Zn, GxZn)		(35,14, 49)		(29,12, 41)		(3.2,1.3, 4.5)

There was no significant relationship between the tissue (shoot, root or seed) Zn concentrations and Zn uptake or translocation values (see Tables 4.2, 4.3 and 4.4). However, significant and positive correlations were found for Zn uptake and translocation results, particularly in the –Zn treatment (Fig. 4.2 and 4.3). For example, the root Zn absorption rate consistently and very highly correlated with shoot Zn uptake (Fig. 4.1, R^2= 0.736, P<0.001) and root-to-shoot translocation rates (Fig. 4.2, R^2= 0.956, P<0.001). These results confirm that wheat genotypes with induced root Zn uptake rates have the ability to translocate more Zn into the shoot at the early vegetative stage (Table 4.3. and 4.4 and Fig 4.1 and 4.2).

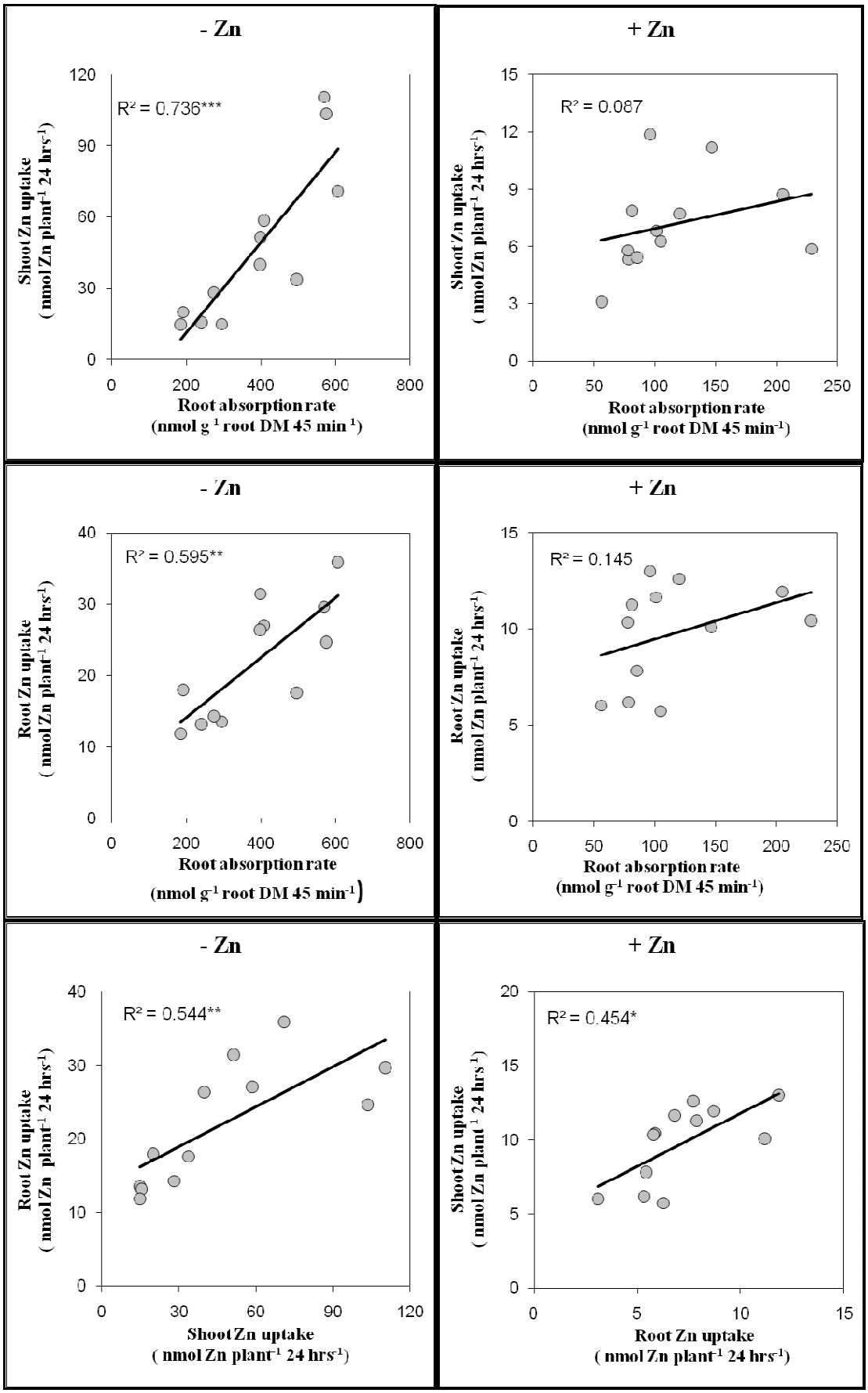

Fig. 4.2. Correlations among shoot Zn uptake, root Zn uptake and root absorption rate.

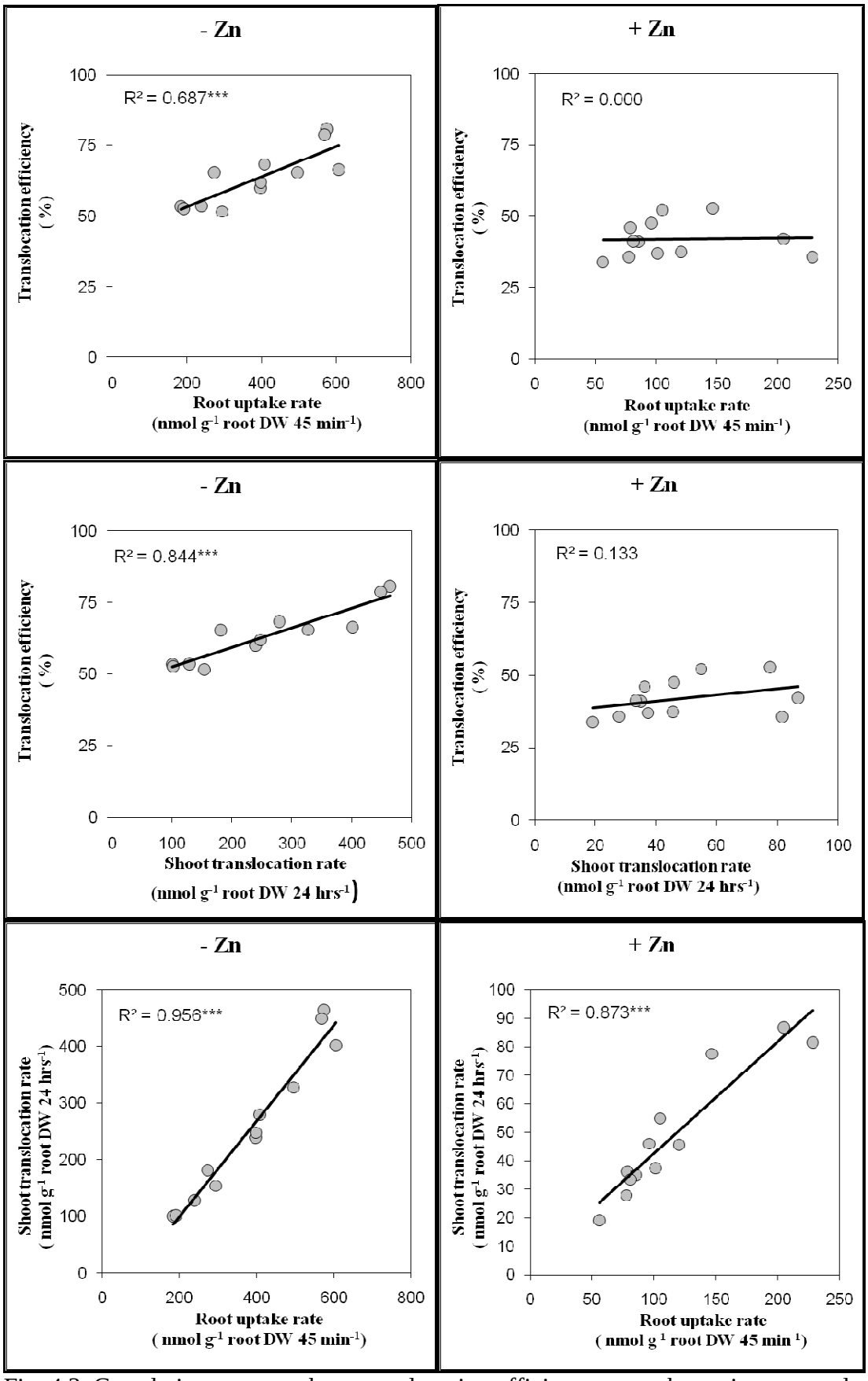

Fig. 4.3. Correlations among shoot translocation efficiency, root absorption rate and shoot translocation rate.

4.2 Zn-65 retranslocation experiment

Dry matter production of experimental plants following 20 days growth with low and adequate Zn supply in nutrient solution is presented in Table 4.5. Shoot dry matter production was slightly reduced with low Zn supply (-Zn) and ranged between 518 mg plant^{-1} (TTD 21) and 1217 mg plant^{-1} (TTD 27) with a mean value of 679 mg plant^{-1} whereas shoot dry matter of control plants (+Zn) ranged between 524 mg plant^{-1} (TTD 21) and 1359 mg plant^{-1} (TTD 27) with a mean value of 774 mg plant^{-1} (Table 4.5). It was noteworthy that two *T. dicoccoides* genotypes had produced the lowest (TTD 21) and highest (TTD 27) shoot dry matter production irrespective of the Zn supply. The average Zn efficiency for shoots was calculated as 89 % and ranged between 103 % (24-39) and 72 % (TD 531) (Table 4.5). For some of the *T. dicoccoides* genotypes (TTD 96, TTD 21, TTD 172, TD 678 and 24-39) low Zn supply had no significant impact on shoot yield, whereas the remaining *T. dicoccoides* genotypes and *T. durum* Desf. genotypes responded to –Zn treatment with a reduction of 10-28% in shoot dry matter production within 20 days of growth in nutrient solution. Statistical analysis confirmed existence of significant differences in shoot dry matter response of genotypes upon different Zn treatments and their interaction.

In contrast to shoot dry matter production, Zn supply had no significant effect on root dry matter production, suggesting that root yield was typically unaffected if not induced with low Zn supply in 20 days. Consequently, average Zn efficiency of roots was calculated as 105%, mainly because some genotypes (e.g. Sarıçanak, TTD 21, TTD 172) had actually produced slightly higher root dry matter in the –Zn treatment. Under the given conditions, *T. dicoccoides* and *T. durum* Desf. genotypes generally responded to the imposed mild Zn deficiency stress (-Zn: 0.05 μM $ZnSO_4$) with a reduction in shoot dry matter production, whereas root dry matter production remained almost unaffected. The changes in dry matter production indicate that a desired mild Zn deficiency was achieved in plants which were further subjected to the Zn mobilization tests. Finally, as mentioned above, the *T. dicoccoides* genotypes TTD 96, TTD 21, TTD 172, TD 678 and 24-39 performed better in terms of dry matter production, whereas cultivated wheats expressed an average Zn efficiency.

Table 4.5 Effect of low (-Zn: 0.05 µM $ZnSO_4$) and adequate (+Zn: 1 µM $ZnSO_4$) Zn supply on shoot and root dry matter production of experimental plants (*Triticum turgidum* L. subsp. *durum* genotypes Sarıçanak 98, and Balcalı 2000 and *Triticum turgidum* L. subsp *dicoccoides* genotypes TTD172, 24-39, TD 153, TD 531, TD 678, TTD 96, TTD 21, TTD 27, TD 536, TD 510) at harvest on 20 days after transplant to nutrient solution. Zinc efficiency values were calculated by the ratio of dry matter production at –Zn to that of +Zn and expressed as percentage.

Genotype	Shoot			Root		
	-Zn	+Zn	Zn efficiency	-Zn	+Zn	Zn efficiency
	(mg plant^{-1})		(%)	(mg plant^{-1})		(%)
Saricanak 98	786	872	90	394	312	126
Balcali 2000	590	647	91	309	294	105
TTD 172	561	565	99	289	253	114
24-39	638	617	103	414	404	102
TD 153	583	750	78	438	426	103
TD 531	633	881	72	468	459	102
TD 678	605	596	101	302	272	111
TTD 96	589	603	98	359	371	97
TTD 21	518	524	99	349	277	126
TTD 27	1217	1359	90	500	531	94
TD 536	587	789	74	405	404	100
TD 510	842	1086	77	432	543	80
Mean	679	774	89	388	379	105
$LSD_{0.05}$(G, Zn, GxZn)	(69 , 28, 98)			(41, NS, 58)		

Changes in tissue Zn concentrations were provided in Table 4.6 along with the seed Zn values to assess the “seed reserve” phenomenon. Shoot Zn concentration in –Zn treatment ranged between 12.3 mg kg^{-1} (Sarıçanak 98) and 21.4 mg kg^{-1} (24-39) with a mean value of 15.5 mg kg^{-1} whereas shoot Zn concentration in +Zn treatment ranged between 71.8 mg kg^{-1} (TTD 96) and 112.2 mg kg^{-1} (TD 678) with a mean value of 86.7 mg kg^{-1} (Table 4.6). As expected, shoot Zn concentrations significantly reduced down to a marginal deficiency level (i.e. 15 mg Zn kg^{-1}) in plants treated with low Zn supply. Although the existence of significant differences in shoot and root Zn concentrations of control plants, no variation was found in the case of low Zn treatment, suggesting that wheat genotypes were experiencing a similar Zn deficiency stress at the time when mobilization test was performed. It was also evident that the inherent variation in seed Zn had no or very little influence on shoot Zn concentrations (see Table 4.6). In

summary, shoot Zn concentration results reflect that the low Zn treatment applied to plants was successful in developing a marginal and evenly distributed Zn deficiency in experimental plants used in the mobilization tests.

Table 4.6 Effect of low (-Zn: 0.05 μM $ZnSO_4$) and adequate (+Zn: 1 μM $ZnSO_4$) Zn supply on shoot and root Zn concentration of experimental plants (*Triticum turgidum* L. subsp. *durum* genotypes Sarıçanak 98, and Balcalı 2000 and *Triticum turgidum* L. subsp *dicoccoides* genotypes TTD172, 24-39, TD 153, TD 531, TD 678, TTD 96, TTD 21, TTD 27, TD 536, TD 510) at harvest on 20 days after transplant to nutrient solution. The initial seed Zn concentrations are also provided to evaluate the possible effect of seed Zn to that of shoot and root Zn concentrations..4.6 Shoot and root Zn concentration comparing two different Zn treatments.

Genotype	Shoot		Root		Seed
	-Zn	+Zn	-Zn	+Zn	
			(mg kg^{-1} DW)		
Saricanak 98	12.3	74.9	17.9	153.3	39.7
Balcali 2000	14.8	95.1	24.4	118.1	35.2
TTD 172	15.0	87.2	32.7	71.7	58.6
24-39	21.4	96.8	25.1	67.1	131.3
TD 153	14.8	94.0	21.2	100.9	45.4
TD 531	16.6	82.6	22.5	83.2	46.6
TD 678	17.2	112.2	28.4	75.7	72.3
TTD 96	15.6	71.8	20.5	78.5	71.8
TTD 21	14.0	87.7	22.8	99.8	68.5
TTD 27	15.4	83.0	20.8	51.2	64.2
TD 536	14.6	74.7	18.1	89.5	50.2
TD 510	14.1	87.0	25.6	56.5	49.7
Mean	15.5	87.3	23.3	87.1	61.1
$LSD_{0.05}$(G, Zn, GxZn)	(6.7, 2.7, 9.4)		(5.4, 2.4, 7.6)		(1.4, - , -)

In leaf retranslocation (mobilization) tests, a large portion of the Zn applied on the leaf tips was retained on the application leaf without been further mobilized. For instance, as much of 82.5 % of the total Zn was retained on the application leaf of control plants (Table 4.7). However, the retained portion of Zn in plants grown with –Zn treatment was substantially lower (i.e. 68.4 %) indicating Zn-deficiency enhanced Zn mobilization towards remainder of shoot and/or roots (Table 4.7). Although the significant differences in relative Zn content (portion of Zn retained in the application leaf) of –Zn and +Zn plants, there was no statistically significant differences among genotypes within –Zn or + Zn treatments (Table 4.7). In control plants, an average of 9.9 % of the Zn taken up by the application leaf was mobilized into shoots and 7.5 % was mobilized into roots. Interestingly, low Zn treatment significantly enhanced Zn mobilization towards the roots but not to the shoots. In average 25.3 % of the total Zn taken up from the application leaf was mobilized into roots in –Zn treatment. Thus, Zn mobilization into roots was found to be 3.4 fold higher under low Zn supply compared to control treatment. Even with substantially higher mobilization rates at -Zn, wheat genotypes expressed no significant differences in Zn mobilization into roots under –Zn or +Zn conditions (Table 4.7). By contrast, statistically significant differences existed in shoot Zn mobilization at a level of genotype, Zn treatment and genotype by Zn interaction (Table 4.7). In other words, genotypes performed significantly different in shoot Zn mobilization ratios. Consequently, *T. dicoccoides* genotypes TTD 27 and 24-39 expressed the highest shoot mobilization ratios whereas TTD 96 and TD 536 had the lowest. The *T. durum* Desf. genotypes Balcalı 2000 and Sarıçanak 98 ranked 6^{th} and 10^{th} in shoot Zn mobilization ratio among a total of 12 wheat genotypes tested.

Table 4.7 Effect of low (-Zn: 0.05 µM $ZnSO_4$) and adequate (+Zn: 1 µM $ZnSO_4$) Zn supply on relative Zn mobilization ratio in shoot, root and application leaf of experimental plants (*Triticum turgidum* L. subsp. *durum* genotypes Sarıçanak 98, and Balcalı 2000 and *Triticum turgidum* L. subsp *dicoccoides* genotypes TTD172, 24-39, TD 153, TD 531, TD 678, TTD 96, TTD 21, TTD 27, TD 536, TD 510) at harvest on 20 days after transplant to nutrient solution.

Genotype	Shoot		Root		Application Leaf	
	-Zn	+Zn	-Zn	+Zn	-Zn	+Zn
			(% $plant^{-1}$ 5 $days^{-1}$)			
Saricanak 98	4.0	8.3	28.7	10.9	67.3	80.9
Balcali 2000	7.5	9.7	27.6	4.2	65.0	86.2
TTD 172	7.9	8.2	21.6	5.8	70.6	86.1
24-39	8.3	15.6	27.8	7.8	63.9	76.6
TD 153	7.6	8.1	22.4	6.4	70.0	85.6
TD 531	4.6	7.1	27.7	4.8	67.7	88.2
TD 678	7.9	8.3	27.4	5.5	64.6	86.2
TTD 96	3.9	8.4	22.1	10.6	74.0	81.0
TTD 21	5.4	11.8	22.3	11.0	72.4	77.2
TTD 27	9.6	10.1	26.7	6.6	63.8	83.4
TD 536	3.8	14.0	19.5	10.4	76.7	75.5
TD 510	5.9	9.9	29.6	6.3	64.5	83.9
Mean	6.4	9.9	25.3	7.51	68.4	82.6
$LSD_{0.05}$(G, Zn, GxZn)	(3.0, 1.2, 4.3)		(NS ,2.6, NS)		(NS, 3.2, NS)	

The relationships among mobilization rates of wheat genotypes at low and adequate Zn supply are shown in Fig. 4.4. Among the wheat genotypes tested, there was a negative and significant correlation between the retained portion of Zn in the application leaf and the mobilized portion of Zn into shoots and roots (Fig. 4.4). Interestingly, the mentioned correlations existed irrespective of the Zn treatments (Fig. 4.4). In contrast, there was no significant relation between shoot and root mobilization ratios (Fig. 4.4) suggesting that these traits are controlled by different physiological mechanisms. The results confirm that the tested wheat genotypes differ significantly in Zn mobilization from application leaf into shoots or roots and *T.dicoccoides* genotypes in general have a higher and exploitable Zn mobilization capacity.

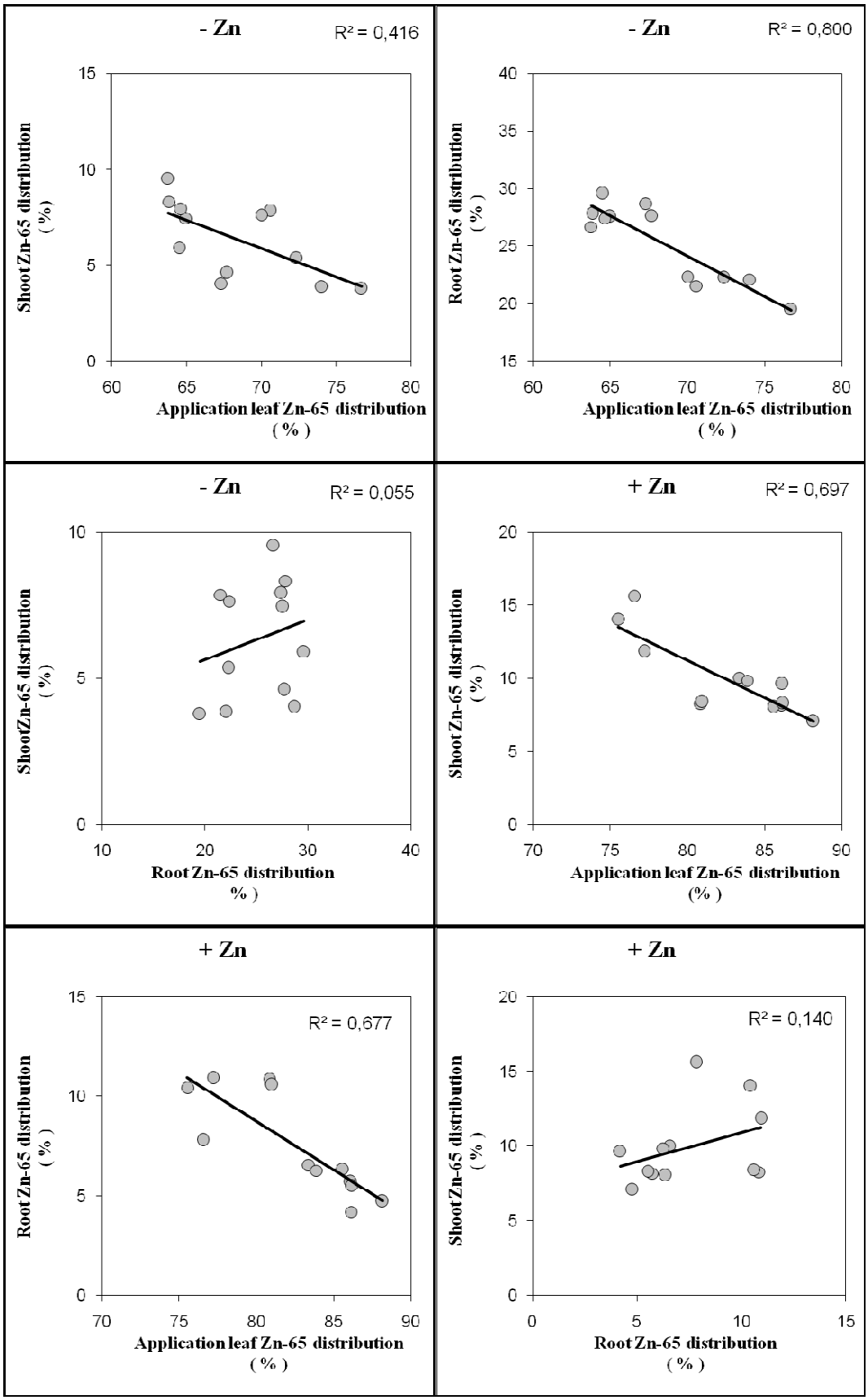

Fig 4.4 Relationships among relative Zn mobilization ratios in shoot, root and application leaf of plants grown with low (-Zn: 0.05 μM $ZnSO_4$) and adequate (+Zn: 1 μM $ZnSO_4$) Zn supply for 20 days in nutrient solution.

5. DISCUSSION

Our knowledge on the molecular and physiological mechanisms involved in Zn accumulation in cereal grains is limited (Cakmak, 2008 and references therein). The large number of studies published during the past few decades yielded no consensus of a single major mechanism that is responsible from grain Zn accumulation. Recently, chromosome 6B was reported as the relevant carrier of genes determining high grain Zn in wild emmer substitution lines (*Triticum turgidum* L. subsp. *dicoccoides*). Wild emmer wheat was proposed as a valuable genetic resource that can be exploited to increase the protein and micronutrient concentration of cultivated wheat, particularly for target micronutrients Fe and Zn (Cakmak et al. 2004). Following studies with wild emmer wheat pinpointed that *Gpc-B1* locus can affect Zn and grain protein concentrations simultaneously (Fahima et al. 2006; Distelfeld et al. 2007). This thesis study focused on understanding the potential of wild emmer wheat by evaluating major physiological mechanisms of Zn uptake, translocation and mobilization using 10 wild emmer wheats along with two cultivated modern wheats (*Triticum turgidum* L. subsp. *durum* Desf.) during the early growth stage of the genotypes Grain Zn accumulation is under influence of various physiological steps starting from roots to seeds (Waters and Sankaran, 2011). At early growth stage, uptake and transport of Zn from growth medium could be very important in Zn accumulation in plant tissue and then re-translocation of Zn from vegetative tissue into growing parts of plants such as shoot tips and seeds (Waters and Sankaran, 2011). In this study, attention has been paid to the uptake and transport process of Zn during the early growth stage of plants.

In the uptake and translocation experiment conducted in this current study, there was no evidence for a superior Zn efficiency of wild emmer wheats over durum wheat cultivars with the exception of TTD 96. Despite of large differences in seed Zn concentrations, there was also no significant GxZn interaction suggesting that all genotypes responded to low Zn supply similarly in dry matter production (Table 4.1). This result seems to be contradictive to previous findings in which under field conditions higher seed Zn content significantly affected plant growth (Yilmaz et al. 1998, Rengel and Graham, 1995a, 1995b). Obviously, seed Zn can determine plant survival and thus growth under field conditions where existence of multiple stress factors are not uncommon (Graham and Rengel, 1993; Cakmak, 2000). However, plants were grown under a controlled environment in in the present study. In addition to a non-stressful environment, the lack of rhizosphere in nutrient solution also may reduce effect of seed Zn reserve on plant growth. Other explanation for the lack of high seed Zn effect on growth under low Zn supply could be related to the fact that the experimental plants under given conditions were not subjected to severe Zn deficiency. As can be seen from the Table 4.1, plants had a slight stress with Zn deficiency (average Zn efficiency was 91 %).

Under low Zn supply, shoot Zn concentrations were in the range of 15 to 20 mg kg^{-1} (Table 4.2) confirming that a targeted mild Zn deficiency was achieved in plants used in the Zn-65 uptake experiments. It was also evident that the significant differences in initial seed Zn concentrations had no effect on shoot or root Zn concentration of the experimental plants (Table 4.2). Average seed Zn concentration was 1.8 fold higher in *T. dicoccoides* genotypes with an extreme of 131.3 mg kg^{-1} (24-39) (Table 4.2).

As expected, low Zn treatment enhanced Zn uptake of durum and wild emmer genotypes (Table 4.3) with an average increase of 6.5 fold in shoot Zn uptake and 2.2 fold in root Zn uptake per plant. Thus, significant differences in shoot Zn uptake occurred in plans grown with low Zn supply (Table 4.3). Sarıçanak 98 expressed an induced Zn uptake to shoot, root and whole biomass in the –Zn treatment, although this induction remained much below the average of wild emmer wheats. Among wild

emmer wheats, TD 531, TTD 27 and TTD 21 expressed an impressive Zn uptake per plant (Table 4.3).

Similar to Zn uptake per plant, Zn uptake and translocation rate per root dry mass were also significantly enhanced by low Zn treatment. Under the control conditions (i.e. sufficient Zn supply) cultivated modern wheats interestingly had higher root uptake and shoot translocation rates than their wild predecessors, whereas in low Zn supply cultivated wheats ranked 4th (Sarıçanak 98) and 8th (Balcalı 2000) out of 12 genotypes. However, under low Zn supply *T. dicoccoides* genotypes TTD 21, TD 531 and TTD 27 had highest root uptake as well as highest shoot translocation rates. These *T. dicoccoides* genotypes also exhibited the highest translocation efficiencies compared to other genotypes. Translocation efficiencies of Sarıçanak 98 and Balcalı 2000 ranked 5th and 12th respectively (Table 4.4). These results indicate that the tested wild and modern genotypes were not distinctly different in their Zn uptake and translocation capacities.

There was also no significant relationship between shoot, root or seed Zn concentrations and Zn uptake or translocation values (see Tables 4.2, 4.3 and 4.4). This is actually a desired result, because any relationship with tissue Zn concentration values can be attributed to a residual artifact such as of seed origin. In contrast, significant and positive correlations were found for Zn uptake and translocation results, particularly in the –Zn treatment (Fig. 4.1 and 4.2). Results indicate that an induced root Zn uptake rate is required for a better translocation to shoot during early vegetative stage in wheat which in turn would determine the extent of grain Zn deposition during grain filling.

In the Zn mobilization (retranslocation) experiment a mild Zn deficiency was exerted by growing the plants with limited Zn supply. There was no variation in shoot Zn concentration of low Zn plants suggesting that the “seed reserve phenomenon” (Rengel, 2001) had no or negligible effect on the results (see Table 4.6). A large portion of Zn applied on the oldest leaf tip was retained without been mobilized. In control plants only 17.5 % of the applied Zn was mobilized form the application leaf to other parts of the plant compared to a mobilization rate of 31.6 % in low Zn treatment (Table

4.7)., indicating that Zn can be transported through phloem channel, especially under low Zn supply with high demand to Zn. An enhanced leaf Zn mobilization rate under limited Zn supply was also reported in rice (Hajiboland et al., 2001) and wheat (Erenoglu et al., 2002) cultivars differing in Zn efficiency. In rice, leaf Zn mobilization was also proposed as an important contributing factor to Zn efficiency (Hajiboland et al., 2001). However, in bread and durum wheat cultivars, Zn mobilization rates were similar and did not correlate to Zn efficiency (Erenoglu et al., 2002). In this thesis study, wild emmer and durum wheat genotypes did not statistically differ in Zn mobilization from the application leaf (Table 4.7). By contrast, statistically significant differences existed in shoot Zn mobilization at a level of genotype, Zn treatment and genotype by Zn interaction (Table 4.7). In other words, genotypes performed significantly different in shoot Zn mobilization ratios. Consequently, *T. dicoccoides* genotypes TTD 27 and 24-39 expressed the highest shoot mobilization ratios whereas TTD 96 and TD 536 had the lowest. The *T. durum* Desf. cultivars Balcalı 2000 and Sarıçanak 98 ranked 6th and 10th in shoot Zn mobilization ratio among a total of 12 wheat genotypes tested. According to literature survey, this is the first report to show significant and exploitable variation in shoot Zn mobilization of wild emmer wheat genotypes. Analysis of data revealed a negative and significant correlation between retained portion of Zn in the application leaf and mobilized Zn into shoots and roots irrespective of Zn treatments (Fig. 4.4). By contrast, no significant relation was detected between shoot and root mobilization rates (Fig. 4.4), suggesting that these traits are controlled by different physiological and genetic factors. Such high translocation capacity for foliar applied Zn through phloem also indicates that plants can respond to foliar Zn application by increasing grain Zn concentration. Accordingly, recently, it has been shown that wheat plants growing under field conditions responded to foliar Zn application by significant increases in grain Zn concentration (Cakmak et al., 2010)

In summary, this thesis study reports a higher and exploitable Zn uptake and mobilization capacity of wild emmer wheats. Under low Zn supply the wild emmer wheats TTD 21, TD 531 and TTD 27 showed highest root Zn uptake and root-to-shoot Zn translocation rates whereas TTD 27 and 24-39 expressed the highest shoot mobilization ratios. Exploitation of Zn uptake, transport and mobilization traits of these genotypes in further breeding studies can greatly contribute to increasing grain Zn

content of cultivated wheats. The wild wheats used in this study were different in their grain Zn concentrations (see Table 4.1); but, as presented in this study, the differences in grain Zn concentration were not related to the differences in root Zn uptake and Zn mobilization (retranslocation) rates of the genotypes during the early growth stage. It is, therefore, important to conduct similar tests on those genotypes during the late growth stage (e.g., after flowering stage).

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APPENDIX A

Heating Block: Ceran 500®

Gamma Counter: Perkin Emler 2480 WIZARD^2 Automatic Gamma Counter

Growth Chamber: DigiTech Hi-tech systems LTD.

ICP (inductively coupled plasma atomic emission spectrometer): Varian VistaPro Axial)

Microwave Oven: CEM MarsXpress

Regular Oven: Memmert beschickung-Loading model 100-800