ADVERSE EFFECT OF HIGH PHOSPHORUS ON PLANT ZINC CONCENTRATION EXPRESSED DIFFERENTLY IN WHEAT PLANTS GROWN IN SOIL AND NUTRIENT SOLUTION

by

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ABSTRACT

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Keywords: Phosphorus, zinc, root, mycorrhiza, wheat

Zinc (Zn) deficiency is a global micronutrient deficiency in agricultural soils. One of the major causes of the widespread occurrence of Zn deficiency is related to its interactions with other nutrients during root absorption, especially with phosphorus (P). In this study, soil and nutrient solution culture experiments were conducted on wheat to examine how increasing P supply affects root Zn uptake and shoot and grain Zn concentrations of plants which were grown under different Zn treatments. Part of the soil experiments has been realized by using autoclaved (sterilized) soils. In the experiments with native soil, there were substantial decreases in shoot and grain Zn concentrations by increasing P applications. Under low Zn supply, increasing P supply also caused decreases in yield and promoted expression of Zn deficiency symptoms. Treatment of the native soil with increasing P supply also resulted in significant depression in mycorrhizal inoculation of roots. In contrast to the results obtained with native soil, Zn concentrations of the plants were slightly affected by increasing P supply when grown in sterilized soil (without mycorrhizae). In case of nutrient solution, enhancements in P supply had either no effect or even stimulated root Zn uptake. Based on these findings, it is suggested that high P itself in nutrient or soil solution has not an adverse effect on chemical solubility or root uptake of Zn in soils. The well-documented reducing effect of increasing P supply on root Zn uptake is most probably related to decline in mycorrhizal activity in rhizosphere.

ÖZET

YÜKSEK DOZDA UYGULANAN FOSFORUN BİTKİ ÇİNKOSU ÜZERİNE OLUMSUZ ETKİSİ TOPRAK VE BESİN ÇÖZELTİSİNDE BÜYÜYEN BİTKİLERDE FARKLI ORTAYA ÇIKMAKTADIR

Emir Ova

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Anahtar sözcükler: Fosfor, çinko, kök, mikoriza, buğday

Çinko (Zn) eksikliği, tarımsal topraklarda üretimi ve kaliteyi olumsuz yönde etkileyen global bir mikroelement eksikliğidir. Çinko eksikliğinin yaygın şekilde görülmesinin en önemli nedenlerinden biri de, kökler yoluyla alımı sırasında çinkonun diğer elementlerle, özellikle de fosfor (P) ile etkileşimidir. Bu çalışmada, toprak ve su kültürü denemelerinde buğdayların artan P ve farklı Zn durumlarında, Zn alımının, yeşil aksam ve dane çinko konsantrasyonlarının nasıl etkilendiği incelenmiştir. Toprak denemelerinin bir bölümü otoklavlı (steril) toprakta gerçekleştirilmiştir. Normal toprakta yürütülen denemelerde artan fosfora bağlı olarak dane ve yeşil aksam Zn derişimlerinde önemli ölçüde düşüşler gözlendi. Düşük Zn koşullarında artan P uygulaması verimde düşüşlere sebep olurken çinko eksikliği semptomlarının şiddetlenmesine neden oldu. Normal toprakta, fosfor oranlarındaki yükseliş, köklerdeki mikoriza gelişimini ciddi şekilde baskıladı. Steril edilmemiş toprakta alınan sonuçlara rağmen, otoklavlı toprakta (mikorizasız durumda), bitkilerin çinko oranlarının yükselen fosfor miktarından çok az etkilendiği gözlendi. Su kültüründe ise, fosforun yükselmesi çinko derişimlerini etkilememiş; hatta bu elementin alınmasını hızladırmıştır. Bu bulguların ışığında, P uygulaması su ya da toprak kültüründe çinkonun kimyasal çözünürlüğüne veya çinkonun kök alımına doğrudan hiç bir olumsuz etkisinin olmadığı söylenebilir. Artan fosforun çinko alımı üzerindeki azaltıcı etkisinin güçlü olasılıkla rizosferdeki mikoriza aktivitesi ile ilişkili olduğu düşünülmektedir.

.

This work is dedicated

To my family, **Kaya**, **Müge** and **Elif**,
Who always stood beside me with their love and peerless support;

To **Melis**, for being so precious to me.

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LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA ..... analysis of variance
B ..... boron
ca ..... circa (approximately)
$CaCO_3$ ..... calcium carbonate
$Ca(NO_3)_2.4H_2O$ ..... calcium nitrate tetrahydrate
$CaSO_4.2H_2O$ ..... calcium sulfate dihydrate
Conc. ..... concentration
Cont. ..... continued
Cu ..... copper
$CuSO_4.5H_2O$ ..... copper sulfate pentahydrate
cv. ..... cultivar
dH_2O ..... distilled water
DTPA ..... diethylenetriamine pentaacetic acid
DW ..... dry weight
Fe ..... iron
Fe-EDTA ..... iron ethylenediamine tetraacetic acid
H_3BO_3 ..... boric acid
HNO_3 ..... nitric acid
HSD ..... honestly significant test
ICP-OES ..... inductively coupled plasma optical emission spectrometry
i.e. ..... id est
K ..... potassium
KCl ..... potassium chloride
K_2SO_4 ..... potassium sulfate
MA ..... mycorrhiza
$MgSO_4.7H_2O$ ..... magnesium sulfate heptahydrate
Mn ..... manganase
$MnSO_4.H_2O$ ..... manganese sulfate monohydrate
N ..... nitrogen
$(NH_4)_6Mo_7O_{24}.4H_2O$ ..... ammonium heptamolybdate (paramolybdate) tetrahydrate

P..... phosphorus

S.....sulfur

Treat. .....treatment

WHO .....World Health Organization

wt..... weight

Zn .....zinc

$ZnSO_4 \cdot 7H_2O$.....zinc sulfate heptahydrate

(A) GENERAL INTRODUCTION

A.1. Zinc is essential for a healthy world

Micronutrient deficiencies have an immense negative influence on human health, and they are reported to affect more than two billion people at a global scale (Cakmak et al., 2010; Welch & Graham, 2004). Micronutrient deficiencies are responsible for the two third of childhood death in the world (Welch and Graham, 2004). Among these micronutrients, Zn has a particular importance. In 2002, World Health Organization (WHO) indicated Zn deficiency as the top fifth factor causing illness and diseases in developing countries (WHO, 2002). In 2008, in Copenhagen Consensus, Zn and vitamin A deficiency problems were identified as top challenges affecting global stability.

Zinc plays a wide range of roles within human body. It is required for the activity of more than 100 enzymes (Hotz and Brown, 2004). Activity and structural stability of about 3000 proteins are affected from Zn (Tapeiro and Tew, 2003). Zinc can also act as a neurotransmitter and play role in signaling (Herschfinkel et al., 2007).

Zinc deficiency causes disturbances in human body functions like physical growth, reproductive and immune system and mental development (Cakmak, 2010). Consequently, a wide range of symptoms appear in case of severe Zn deficiency such as increased vulnerability to infectious diseases, stunting, delayed bone maturation, impaired sexual and cognitive development, increased morbidity and mortality (Welch and Graham 2004; Gibson et al., 2008). Zinc deficiency is estimated to be responsible for 14.4% diarrhea deaths, 10.4% malaria deaths and 6.7% pneumonia deaths within children between 6 months and 5 years old (Black, 2008). These reports highlight that Zn plays irreplaceable roles within human physiology and is primarily required to maintain a healthy life.

A.2. Underlying mechanisms triggering zinc deficiency

Zinc deficiency in human populations is mostly caused by low dietary intake; but sometimes, although its uptake might be sufficient, there are other factors preventing the absorption and cellular utilization of Zn such as the existence of compounds like phytate and phenolics in the diet (Sandberg, 2002; Gibson et al., 2008). In addition, physiological Zn requirement is changing at different stages of human development (infancy, pregnancy etc.) (Gibson and Ferguson, 1998).

The reasons of Zn deficiency in well-developed and developing countries are distinctive. In rich countries Zn deficiency emerge by low energy intake that is sourced from concerns towards body weight and wrong food choices (Houston & Summers & Soltesz, 1997). People who consume predominantly plant-based food are also subject to higher risk of Zn deficiency because higher concentrations of phytic acid in plant based foods lower bioavailability of Zn in their diet (Hunt, 2003). In developing countries, food consumption switches from high Zn containing expensive animal-based foods to cheaper plant based foods which are poor Zn sources (Murphy and Allen, 2003). Cereals, especially wheat, play a particular role in the nourishment of the human populations living in developing world. In many countries which are located in Central Asia and Middle East, approximately 50% of daily calories come from wheat consumption, and this ratio may reach up to 70% in rural areas (Cakmak, 2008). Cereals are known to be inherently low in micronutrients and rich in anti-nutrients such as phytic acid which reduces the utilization of Zn in human body (Welch and Graham, 2004). Today, most of the cereal-cultivated soils are very low in plant available (chemically soluble) Zn which further reduces concentration of Zn in cereal grains and contribute to widespread occurrence of Zn deficiency in human populations as shown in India, Pakistan, Turkey and China (Alloway, 2004; Cakmak, 2008).

A.3. Solutions to minimizing zinc deficiency in human populations

There are several approaches to mitigate severity of Zn deficiency-related health problems in human beings. First thing that was suggested was the Zn-supplementation of population who are primarily subject to high risk. Although Zn-supplementation is a successful strategy, the target population is, however, too large to reach every individual with

additional supplementation, and the economic burden of this approach would be too high to meet the demand (Pfeiffer and McClafferty, 2007). Alternatively, it has been suggested that biofortification of the staple crops that are widely consumed at a global scale represents the most effective strategy (Underwood, 2000, Bouis, 2003; Pfeiffer and McClafferty, 2007). It was also suggested that biofortification can be an efficient tool on preventing deaths and morbidity due to Zn deficiency in India (Stein et al., 2007). On the basis of these studies, biofortification has a major potential on alleviating micronutrient deficiencies in a cost-effective way. Besides, every member of target population may easily access to these crops.

Recently it has been shown that nitrogen (N) nutritional status of plants is an important player in agronomic biofortification of food crops with Zn or Fe. It was shown that grain Zn and Fe accumulation is enhanced by increased N availability in growth media or increased tissue N (protein) concentrations (Aciksoz et al., 2011; Kutman et al., 2012). By contrast, increased P supply lowered Zn levels within plant tissues (Singh et al., 1986). In order to fully utilize the tool of agronomic biofortification, the interactions of N and P with Zn should be well understood.

A.4. Roles of zinc in plants

Zinc has numerous critical roles in biological systems. It is found in the structure of DNA binding proteins, and therefore, Zn is in a close association with DNA, RNA metabolism, cell division and protein synthesis (Coleman, 1992). Several reports indicate existence of almost 2800 proteins in biological systems which require Zn for their functional integrity and structural stability (Andreini et al. 2009).

In plant systems Zn deficiency can cause severe reductions in biomass and protein levels accompanied with amino acid accumulation, suggesting that protein synthesis is severely impaired under such conditions (Cakmak et al. 1989). Many of the crucial enzymes like alcohol dehydrogenase, carbonic anhydrase, superoxide dismutase RNA polymerase, require Zn to maintain their function (Marschner, 2012). In case of Zn deficiency, production of reactive oxygen species is also enhanced but the activity of an antioxidant enzyme, superoxide dismutase dramatically reduced (Cakmak and Marschner, 1988). Symptoms like necrotic spots and chlorotic leaf appearance seem to be caused by Zn-deficiency induced generation of free radicals (Cakmak, 2000). Plants under low Zn supply could not cope with

toxic free radicals due to weak antioxidative defense mechanism such as low superoxide dismutase which detoxifies superoxide radical. The activities of catalase and ascorbate peroxidase (which detoxify hydrogen peroxide) are also reduced, probably due to the decline in protein synthesis, and therefore plants became more vulnerable to oxidative damage under Zn deficiency (Cakmak, 2000).

It is therefore important to maintain an adequate Zn supply to crop plants to avoid yield depressions. In field experiments conducted on a Zn deficient soil of Central Anatolia, additional Zn application increased grain yield by 2.6 fold which emphasizes importance of Zn nutrition of crop plants (Yilmaz et al., 1997). Having high Zn in seeds has also important benefits for better growth and yield. Usage of Zn-enriched seeds provided improved abiotic stress tolerance, increased resistance to diseases and enhanced seed viability (Cakmak, 2008). These results indicate that increasing Zn levels of plants and seeds has very high positive impacts on both crop production and human nutrition and health.

As indicated above, Zn nutrition of plants is greatly affected by various nutrients such as N and P. Generally, improving N nutritional status of plants grown on potentially Zn-deficient soils greatly contribute to better Zn uptake and accumulation and avoid occurrence of Zn deficiency stress (Kutman et al., 2011). However, in case of high P supply, root uptake of shoot concentrations of Zn are adversely affected causing expression of Zn deficiency in plants (Nichols et al., 2012; Marschner, 2012). Below a separate section is given illustrating the interactions between P and Zn.

A.5. Role of high phosphorus supply in zinc nutrition of plants

Phosphorus represents an important macronutrient and has diverse of important functions in plant cells. Phosphorus is found in the structure of nucleic acids, DNA, RNA, ATP and phospholipids and affects very positively photosynthesis, use of photoassimilates for growth and development, transfer and storage of energy within plants (Marschner, 2012). Therefore, it is not surprising why rapidly expanding leaves contain very high P concentrations which are used energy metabolism and structural and functional integrity ribosomal RNA (Suzuki et al., 2001). Phosphorus also plays important roles in physiological process like signaling (Karthikeyan et al., 2007), cell division (Assuero et al., 2004) and leaf expansion (Clarkson et al., 2000).

It is known that most of P in plants is stored in the form of phytate, especially in seeds (Lott et al., 2000) and phytate has high capacity to bind strongly to Zn and Fe (Wang et al., 2008). Cereal and legume seeds are rich in phytate; up to 85 % of seed P is stored in form of phytate in seeds (Cakmak, 2008). Therefore, plant-based diets (particularly cereal-based diets) with high concentrations of phytate are very risky for micronutrient nutrition of human populations and may result in Zn deficiency by reducing absorption of Zn through intestines in monogastric animals (Welch et al., 1974) and humans (Kumar et al., 2010).

High phosphorus has been also reported to be antagonistic with Zn nutrition of crop plants. Plants grown in high P supply usually contain lower amount of Zn in tissues, and under low Zn supply, plants absorb and accumulate huge amounts of P which may be even toxic to plant cells (Cakmak and Marschner, 1986; Loneragan and Webb, 1993). High P accumulation in plant cells is suggested to be one reason for the P-induced Zn deficiency due to proposed P-Zn precipitation in plant tissues (Cakmak and Marschner, 1987). However, there is contradicting information in the literature about P-Zn precipitation as well.

There are large number of controversial results in literature regarding the impact of high P on root Zn uptake and tissue Zn concentrations of plants. A decline in plant Zn was found by increasing P supply in wheat and barley plants (Singh et al., 1986; Zhu et al., 2001, Li et al., 2003) while in cotton, potatoes and maize plants exhibited Zn deficiency symptoms with higher P supply, although tissue Zn levels didn't change by increasing P treatments (Cakmak and Marschner, 1987; Barben et al., 2010; Nichols et al., 2012). According to Nichols et al (2012), root Zn concentrations are increased by increasing P supply in maize plants. It seems that growth conditions (soil or nutrient solution) play an important role in such controversial results.

There are several mechanisms discussed in the literature as possible explanations for the P-Zn interactions in plants: Zinc exists in the form of Zn^{2+} and P exists in the form of $H_2PO_4^{1-}$ or HPO_4^{2-}. As a result of ionic interactions, P and Zn can be precipitated within soil or plant tissue causing biologically unavailable Zn to plant cells (Loneragan et al., 1979). According to this idea, P has high possibility to lower physiological availability of Zn to metabolic functions in plants. Previously, Cakmak and Marschner (1987) showed that increasing P supply and thus increase in tissue P concentrations did not affect the total concentration of Zn, but greatly diminished the total amount of water soluble Zn which indicates existence of a possible chemical interaction between Zn and P (probably a P-Zn precipitation). In a previous study, Youngdahl et al (1977) showed that increasing P supply

enhanced the amount of Zn in root cell walls, especially in the pectate fraction of cell walls in maize plants. This effect of high P has been discussed as an important adverse effect of high P on leaf Zn concentrations in plants. Very recently, Nichols et al (2102) also showed that P increases root Zn concentration of plants. A slower Zn translocation rate from root to shoot in the presence of higher P supply was also considered as an important contributory factor to the interaction between P and Zn (Alloway; 2004; Khanif and Saleem, 2013).

It is well-known that shoot to root ratio of plants increases by high P applications (Marschner, 2012). Plants with high P tend to produce relatively less root and higher shoot biomass. Reduction in root growth by high P supply would mean less exploration of soil and consequently less root uptake of Zn. This impact of high P might be important in potentially Zn-deficient soils. In addition, with higher P treatment plants tend to produce more shoot biomass which may also cause dilution of Zn in tissue and thus occurrence of Zn deficiency (Zhu et al., 2001; Marschner, 2012).

Phosphorus and Zn interaction was also discussed in respect to mycorrhizal infection of roots which is believed to have marked effects on root Zn uptake (Kothari et al., 1991 and Smith and Read, 2008). As discussed in more detail below, mycorrhiza activity is very low under high P conditions so that it cannot contribute to root Zn uptake (Ryan et al., 2008; Marschner, 2012).

A.6. Mycorrhiza: a critical component of PxZn interaction

Mycorrhiza is widespread throughout the agricultural soils and it is known to be in close association with plant roots. Around 80 % of monocotyledonous and dicotyledonous plants can interact with mycorrhiza (Smith and Read, 2008). Fungus can provide enhanced access to nutrients for the host plants through its hyphae in bulk soil where normally roots cannot reach. In return, plants provide photoassimilates to support the growth of mycorrhiza. However, the relation can shift from mutualistic to parasitic, by factors, such as the availability of P, light intensity or host species (Marschner, 2012). For example, in case of abundance of nutrients in soil where plants no longer needs mycorrhizal association, the host still provides carbon supply for the fungus, as a result the relation becomes parasitic.

There are two main mycorrhiza groups as endomycorrhiza and ectomycorrhiza. The actual difference between two groups was that ectomycorrhiza does not penetrate host cells

and remained in the intercellular space but endomycorrhiza can grow intracellular, in a way that membranes of host and fungus cells are in direct touch (Marschner, 2012). Endomycorrhiza comprises the subgroup arbuscular mycorrhiza (MA) which can form the structure of arbuscules where the exchange of matter between fungus and host takes place (Smith and Read, 2008).

AM fungi are also found in association between wheat plants and hyphae formation of mycorrhiza can significantly increase root-soil interface area and contribute to nutrient uptake like P, Zn and Cu (Marschner, 2012). Phosphorus uptake in mycorrhizal plants can be 2-3 times higher comparing to non-mycorrhizal plants (Tinker et al., 1992). In a study carried out with *Triticum aestivum* arbuscular mycorrhiza contributed to the P uptake of plants by 50% and in the same paper it was indicated that increasing P application decreased colonization density in host plants (Li et al., 2006). Colonization density is also negatively affected by intensive soil disturbance or widespread practices like tillage (Jasper et al., 1989; Garcia et al., 2007). Mycorrhiza has the potential of increasing Zn levels of plants remarkably. In wheat plants the positive effect of mycorrhiza on Zn concentrations and negative impact of P on plant Zn composition was demonstrated before (Ghasemi-Fasaei and Mayel, 2012). Considering all the information above, mycorrhiza seems to be an important factor affecting the extent of P and Zn interaction.

A.7. Objectives

Published data indicates existence of many contradictory reports about P-Zn interaction in terms of role of P in reducing root Zn uptake and leaf or shoot Zn concentrations. It seems likely that these controversial results are related very much to the growth conditions such as use of soil or nutrient solution culture. In addition, in those experiments mentioned above, role of mycorrhizae was not systematically tested and the effects found were not reported with or without mycorrhizae. In the framework of this thesis, we have first used a native soil and investigated the impact of increasing P supply (e.g., three P rates) on i) shoot and grain yield, ii) shoot and grain concentrations of Zn and other elements and iii) development of leaf Zn deficiency symptoms. These tests have been realized on a Zn deficient soil with low and adequate Zn applications. Additionally, impact of increasing P supply on mycorrhizal infection of roots was studied under given conditions. The results obtained were presented in

the Chapter-I. In the second Chapter, similar experiment has been established by using native and autoclaved (sterilized) soil in order to examine impact of mycorrhizae on plant growth and yield and concentration of Zn and other nutrients in leaves and grain.

In the Chapter-III, we studied interaction of P and Zn in nutrient solution experiments by using same wheat cultivar. In this nutrient solution experiment, special attention has been given to the effects of increasing P supply on root Zn uptake, development of Zn deficiency symptoms and shoots Zn concentrations.

Based on the experiments of the Chapters described we will clarify following major question: what is impact of increasing P supply on root Zn uptake and shoot Zn accumulation in soils with and without sterilization (e.g., with or without mycorrhizae) and in a nutrient solution (without soil). We hypothesized that the inhibitory role of high P on Zn uptake is not related to any direct (ionic) interaction between P and Zn, as proposed several times in literature. The inhibitory role of high P in Zn uptake and in Zn accumulation in plant tissues is rather related to high P-reduced mycorrhizal infection of roots.

(B) GENERAL MATERIALS AND METHODS

In all experiments that were conducted under this thesis, a Turkish bread wheat variety (*Triticum aestivum* cv. Adana99) was used.

B.1. Plant Growth Facilities

B.1.1. Greenhouse

The greenhouse where all soil trials and one hydroponic experiment was carried out is located in Sabanci University Campus, Tuzla, Istanbul. The greenhouse contains a heating and an evaporative cooling system.

B.1.2. Growth Chamber

One of the hydroponic (nutrient solution) experiments was conducted in a growth chamber where climatic factors (light/dark regime: 16/8 h; temperature (light/dark): 24˚C/22˚C; relative humidity (light/dark): 60%/70%; photosynthetic flux density: 400 µmol m^{-2} s^{-1}) were controlled.

B.2. Soil Culture

The soil used in this experiment was transported from Central Anatolia, Eskisehir. It is characterized as calcareous (18% $CaCO_3$) and alkaline (pH 8 in dH_2O) soil with clay-loam texture and low organic matter (1.5%). According to the DTPA analysis method which was

conducted according to Lindsay and Norvel (1978), soil contained approximately 0.1 mg kg^{-1} of extractable Zn, which is in deficiency range. The rates of Zn and P applications were presented in the related chapters.

B.3. Solution Culture

Seeds were sown and germinated in perlite that was watered with deionized water for 5-6 days and kept at room temperature. After that, seedlings were transferred to 3 L plastic pots with aerated following nutrient solution: 2 mM $Ca(NO_3)_2.4H_2O$, 0.7 mM K_2SO_4, 0.75 mM $MgSO_4.7H_2O$, 0.1 mM KCl, 100 µM Fe-EDTA, 1 µM H_3BO_3, 1 µM $MnSO_4.H_2O$, 0.2 µM $CuSO_4.5H_2O$, and 0.01 µM $(NH_4)_6Mo_7O_{24}.4H_2O$.

The Zn and P treatments were made in the form of $ZnSO_4.7H_2O$ and $Ca(H_2PO_4)_2$, respectively. In order to keep Ca levels of the low P-pots constant, $CaSO_4.2H_2O$ was added at according amounts. Nutrient solution were constantly aerated and replaced every 3-4 days.

B.4. Harvest

At harvest, plants samples collected were washed several times with deionized water and then dried at 60°C. Grains were automatically separated from husk using thresher machine. Dried samples were then weighed for determination of dry matter production and thereafter subjected to nutrient analysis.

B.5. Element Analysis

The washed and dried plant samples were ground in an agate vibrating cup mill (Pulverisette 9; Fritsch GmbH; Germany) for analyses of mineral nutrients. Dried and ground samples (ca. 0.4 g of grains and 0.2 g of shoot or root) were digested with 2 ml 30% H_2O_2 and 5 ml 65% HNO_3 using a microwave system (MarsExpress; CEM Corp., Matthews, NC, USA). Digestion solutions were topped up to 20 ml by adding double-distilled water. Element

concentrations of K, P, S, Mg, Ca, Zn, Fe, Cu and Mn were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) (Vista-Pro Axial, Varian Pty Ltd, Mulgrave, Australia). Certified standard reference materials were used to check the values obtained in ICP-OES. Reference materials were obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA).

B.6. Calculations

In order to calculate the mineral content of plants, element concentrations were multiplied with the total biomass of the tissue of interest (i.e. grain, shoot content).

Harvest index (%) was calculated as [grain yield/ (straw DW + spike DW)] x100.

B.7. Statistical Analysis

In the calculation of statistical analyses, JMP software program was used. Analysis of variance (ANOVA) was utilized to identify the significance of applications. The significance of the differences between means was calculated by Tukey's honestly significant difference (HSD) test at the level of ($p \leq 0.05$).

CHAPTER 1

INCREASING PHOSPHORUS APPLICATION REDUCES PLANT ZINC CONCENTRATIONS AND INDUCES ZINC DEFICIENCY SYMPTOMS AT LOW ZINC SUPPLY

1.1. Introduction

Increasing published evidence is available showing that P has an antagonistic effect on root Zn uptake and Zn nutritional status of plants (Loneragan et al., 1982; Cakmak and Marschner, 1987; Nicholas et al., 2012). There are, however, many contradicting information in the literature about the nature of the PxZn interaction. In one field experiment carried out with wheat, increases in P application led to remarkable reduction in tissue Zn concentration and impaired mycorrhizal infection of roots (Singh et al., 1986). There are other papers indicating a clear decline of tissue Zn levels with respect to increasing P supply in wheat and barley (Zhu et al., 2001, Li et al., 2003). In one study carried out in oilseed rape there was no reduction in plant Zn concentrations by increasing P treatments (Lu et al., 1998). Singh et al. (1988). Gianquinto et al. (2000) reported that decreases in tissue Zn concentrations of *Phaseolus vulgaris* were related to a dilution effect caused by an increase in shoot growth associated by a high P supply. In case of a nutrient solution experiment, leaf Zn concentrations of cotton plants did not fall by increasing P supply, although increasing P supply stimulated occurrence of Zn deficiency symptoms in cotton (Cakmak and Marschner, 1987). According to Cakmak and Marschner (1987), under low Zn supply enhanced P supply causes substantial increases in shoot P concentrations which, in turn, reduce physiological availability or utilization of Zn in tissue although high P treatments did not affect the total amount of Zn in plant tissues. Phosphorus-induced reductions in physiological availability of Zn in tissue have been demonstrated by low levels of water soluble Zn in plants exposed to high P supply (Cakmak and Marschner, 1987).

In another solution culture experiment that was carried out with maize, Nichols et al. (2012) reported that upon increasing P treatments, shoot Zn levels were not changed but root Zn concentrations were elevated which indicates that high P supply causes retention of Zn in root tissue. In a previous work, Youngdahl et al (1977) suggested that root cell walls have a very high Zn binding/fixing capacity when they grown in a growth medium with high P supply.

Number of reports are available indicating mycorrhizae very positively contributes to root uptake of Zn, and root infection with mycorrhizae is limited by increasing P treatments (Kothari et al., 1991; Ryan et al., 2008; Marschner, 2012).

The results mentioned above indicate that in evaluation of the PxZn interaction a special attention should be given to the growth conditions. It appears that the nature of the PxZn interaction greatly differ depending on the use of soil or nutrient solution culture studies. In this chapter, effect of increasing P supply on growth, nutrient concentrations and severity of Zn deficiency symptoms was studied in a Zn-deficient soil with low and adequate Zn supply. Plant roots were also examined for the level of mycorrhizal infection.

1.2. Materials and Methods

1.2.1 Experimental Design

This study was designed as a factorial experiment with 5 independent replicates.

Each plastic pot was filled with 3.1 kg of soil and supplied with 300 mg N in the form of $Ca(NO_3)_2.4H_2O$ with 25 mg S in the form of K_2SO_4 per kg of soil and then soil was homogenously mixed. Three different rates of P were used as following: 15 mg kg^{-1}, 60 mg kg^{-1} and 180 mg kg^{-1} applied in the form $Ca(H_2PO_4)_2$, and two different Zn rates were used: 0.2 mg kg^{-1} (low Zn supply) and 5 mg kg^{-1} (adequate Zn supply) in the form of $ZnSO_4 \cdot 7H_2O$.

Twelve seeds were sown in each pot and upon emergence the seedling numbers were thinned to seven. Two plants were harvested at heading after 66 days of growth for mycorrhizal infection analysis, and 2 plants were harvested after 81 days of growth (e.g., at early milk stage) to study dry matter production and nutrient accumulation (especially Zn) .

The remaining plants were harvested at maturity. On the 52nd day every pot was supplied with 100 mg kg^{-1} of additional N in the form of $Ca(NO_3)_2.4H_2O$ to avoid any risk with N deficiency. Pots were watered daily with deionized water.

Harvested plants were dried, weighed and analyzed for element composition as indicated in "General Materials and Methods".

1.2.2 Mycorrhizal Infection Analysis

In the first harvest 2 plants were isolated from the pots in order to analyze the degree of the mycorrhizal infection according to Giovanetti and Mosse (1980). Roots isolated were washed with deionized water and then 2.5-3 cm of root tips were cut and preserved in ethanol until analysis. The roots stored in ethanol were first immersed in 10% KOH and kept at 65^{o}C for 1 hour. Then, KOH solution replaced with 10% HCl and kept at 15 min at 65^{o}C. After that, HCl and 0.05% Trypan solution added and incubated for 25 min at 65^{o}C. Finally, roots were preserved in lactic acid for the tests. Approximately, 10 root pieces were arranged under microscope, each with a size of ca. 1 cm of every sample and rated on a scale from 1 to 10 depending on mycorrhizal infection.

1.3. Results

In the first experiment, wheat plants were first grown until early milk stage (81 days-old) and then harvested for analysis of dry matter production and shoot concentrations of nutrients. As presented in Table 1.1, increasing P supply enhanced shoot dry matter production both under low Zn supply and adequate Zn supply. The increases in growth were particularly high from low to adequate P supply. At a given P supply, increasing Zn application increased dry matter production only in case of high P supply. The plants grown at high P and low Zn rates showed Zn deficiency leaf symptoms had a reduced growth (Fig. 1.1). Low Zn plants with high P rate had very distinct necrotic spots on leaves, and increasing P supply resulted in depression in growth of wheat plants at low Zn supply (Fig. 1.1).

Table 1.1: Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P rates on shoot dry matter production of 81-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants grown with low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}) Zn supply under greenhouse conditions.

Zn Treatment	P Treatment	Shoot DW (g $plant^{-1}$)
Low	Low	3.3 ± 0.5
	Adequate	5.1 ± 0.4
	High	5.1 ± 0.4
Adequate	Low	3.4 ± 0.3
	Adequate	5.0 ± 0.4
	High	5.7 ± 0.7

Shoot DW $HSD_{0.05}$ (Zn; P; ZnxP): (*n.s*; 0.5; *n.s*)

Fig 1.1: Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P applications on growth of 81-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants grown with low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}) Zn supply under greenhouse conditions.

At the lowest P supply, plants had very low dry matter production irrespective of Zn supply which indicate existence of P deficiency stress in plants (Table 1.1)

The most important finding with this experiment was the concentrations of Zn and P in the experimental plants. At both low and adequate Zn supply, increase in P application rate very significantly reduced shoot Zn concentration of plants (Table 1.2). At low Zn supply, shoot Zn concentration was reduced from 11 mg kg^{-1} to 6 mg kg^{-1} and at adequate Zn supply, shoot Zn was reduced from 41 mg kg^{-1} to 26 mg kg^{-1} by increasing P supply. These decreases in shoot Zn by P are very substantial, and in case of low Zn supply, plants started to show Zn deficiency symptoms as shown in Fig. 1.1. The Zn concentration found under high P and low Zn supplies was 6 mg kg^{-1} that is extremely low and accordingly plants showed very severe Zn deficiency symptoms (Fig. 1.1).

As expected, there was a clear increase in shoot P concentration by increasing P supply. This effect was found to be very similar under both low and high Zn supply (Table 1.2).

Table 1.2: Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P applications on shoot concentrations of Zn and P of 81-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants grown with low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}) Zn supply under greenhouse conditions.

Zn Treatment	P Treatment	Zn Concentration (mg kg^{-1})	P Concentration (% dry wt)
Low	Low	11 ± 1	0.15 ± 0.01
	Adequate	7 ± 1	0.24 ± 0.04
	High	6 ± 0	0.32 ± 0.00
Adequate	Low	41 ± 2	0.15 ± 0.01
	Adequate	28 ± 1	0.20 ± 0.02
	High	26 ± 1	0.31 ± 0.02

Zn Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (1; 1; 2)

P Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (0.01; 0.02; *n.s*)

At grain maturation, plants were harvested to determine grain yield and straw dry matter yield. The results are presented in Table 1.3. As found in shoot dry matter production at early milk stage, increasing P supply improved both grain yield and straw dry matter. At

the lowest P supply, yield values were very significantly reduced due to severe P deficiency. It was interesting to notice that at low Zn supply, straw dry matter yield progressively increased by increased P application from adequate to high level; but grain yield showed a decrease (Table 1.3). In case of adequate Zn supply, increases in P supply improved both grain yield and straw yield.

These results indicate that generative growth is more sensitive to low Zn supply than the vegetative growth. Accordingly, the harvest index value showed a decrease by increasing P supply under low Zn treatment while at adequate Zn, harvest index increased by increase in P supply (Table 1.3).

Table 1.3 Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P applications on grain yield, straw dry matter yield and harvest index bread wheat (*Triticum aestivum* cv. Adana99) plants grown until grain maturation with low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}) Zn supply under greenhouse conditions.

Zn Treatment	P Treatment	Grain Yield (g $plant^{-1}$)	Straw Dry Matter (g $plant^{-1}$)	Harvest Index (%)
Low	Low	2.6 ± 0.2	2.3 ± 0.2	46 ± 2
	Adequate	4.2 ± 0.3	4.2 ± 0.4	43 ± 3
	High	3.9 ± 0.2	4.7 ± 0.6	40 ± 3
Adequate	Low	3.0 ± 0.3	2.6 ± 0.3	46 ± 2
	Adequate	5.2 ± 0.4	4.1 ± 0.1	47 ± 2
	High	6.4 ± 0.8	4.4 ± 0.1	49 ± 4

Grain Yield $HSD_{0.05}$ (Zn; P; ZnxP): (1.0; 1.4; 2.5)

Straw Dry Weight $HSD_{0.05}$ (Zn; P; ZnxP): (*n.s*; 1.1; *n.s*)

Harvest Index $HSD_{0.05}$ (Zn; P; ZnxP): (2; *n.s*; 5.)

There were dramatic reductions in grain concentration of Zn by increasing P supply, especially in case of low Zn treatment (Table 1.4). Grain Zn was declined from 33 mg kg^{-1} to 9 mg kg^{-1} by increasing P supply to the low Zn plants. The reduction in grain Zn of the adequate Zn plants was also very significant (e.g., from 60 to 34 mg kg^{-1}). As expected, applying high Zn resulted in higher grain Zn concentration at a given P supply.

Grain P concentrations showed progressive increase with increasing P supply at both low and high Zn supply. The plants with low Zn tended to contain more P in grain, especially at lower P treatments (Table 1.4).

In contrast to Zn, grain concentrations of other nutrients were either not changed or even increased by increasing P supply both at low and adequate Zn supply. As presented in Table 1.5, increases in P supply, grain concentrations of K and Mg showed a clear increase in both Zn treatments which indicate that the inhibitory effect of high P on grain Zn is a specific effect.

Table 1.4: Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P applications on grain concentrations of Zn and P of bread wheat (*Triticum aestivum* cv. Adana99) plants grown until grain maturation with low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}) Zn supply under greenhouse conditions.

Zn Treatment	P Treatment	Zn Concentration (mg kg^{-1})	P Concentration (% dry wt)
Low	Low	33 ± 5	0.29 ± 0.02
	Adequate	13 ± 0	0.39 ± 0.01
	High	9 ± 1	0.44 ± 0.02
Adequate	Low	60 ± 2	0.24 ± 0.01
	Adequate	47 ± 5	0.35 ± 0.04
	High	34 ± 3	0.44 ± 0.02

Zn Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (3; 4; *n.s*)

P Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (0.01; 0.02; *n.s*)

Table 1.5: Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P applications on grain concentrations of K and Mg of bread wheat (*Triticum aestivum* cv. Adana99) plants grown until grain maturation with low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}) Zn supply under greenhouse conditions.

Zn Treatment	P Treatment	K (%)	Mg (%)
Low	Low	0.35 ± 0.01	0.13 ± 0.01
	Adequate	0.38 ± 0.01	0.15 ± 0.00
	High	0.40 ± 0.01	0.16 ± 0.00
Adequate	Low	0.32 ± 0.01	0.12 ± 0.00
	Adequate	0.35 ± 0.02	0.15 ± 0.01
	High	0.37 ± 0.01	0.16 ± 0.01

K Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (0.01; 0.01; *n.s*)

Mg Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (*n.s*; 0.01; 0.01)

Similarly also grain concentrations of micronutrients such as Fe, Mn and Cu were not affected from increasing P treatments; even, there was significant increases especially with Mn and Fe (Table 1.6). It is of great interest that enhancements in P rates reduces substantially grain Zn concentrations while grain Fe and Mn are markedly increased.

Table 1.6: Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P applications on grain concentrations of Fe, Cu and Mn of bread wheat (*Triticum aestivum* cv. Adana99) plants grown until grain maturation with low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}) Zn supply under greenhouse conditions.

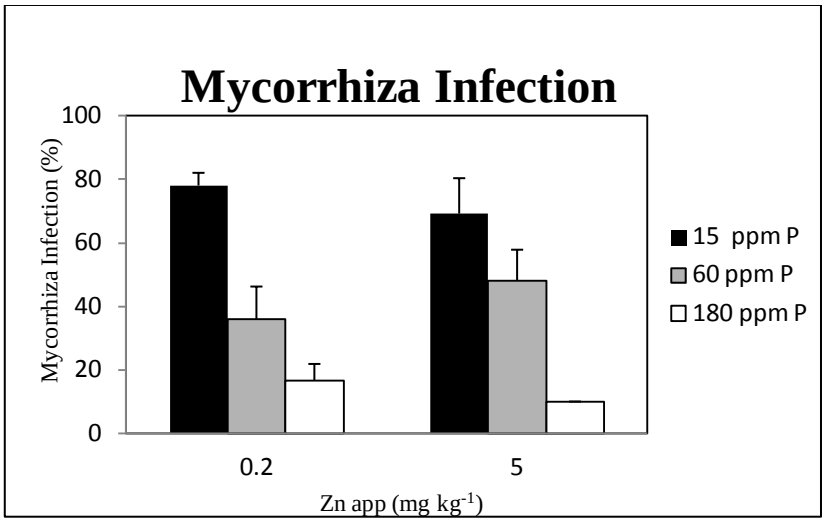
Zn Treatment	P Treatment	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})
Low	Low	24 ± 0	6 ± 0	39 ± 2
	Adequate	29 ± 2	7 ± 0	54 ± 2
	High	31 ± 2	7 ± 0	68 ± 4
Adequate	Low	25 ± 1	7 ± 0	36 ± 3
	Adequate	33 ± 2	7 ± 0	49 ± 1
	High	34 ± 3	6 ± 0	53 ± 4

Fe Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (2; 3; *n.s*)

Cu Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (*n.s*; *n.s*; 1)

Mn Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (2; 4; 7)

When plants were at the beginning of flowering, roots were isolated to measure mycorrhizal infection. The results obtained are presented in Fig. 1.2. Mycorrhizal infection of roots dropped significantly with P application irrespective of the Zn treatment. When P supply was high, mycorrhizal propagules could not form successfully and fungal development was severely impaired (Fig 1.2).



$HSD_{0.05}$ (Zn; P; ZnxP): (*n.s*; 21; 36)

Fig 1.2: Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P, with two different Zn treatments as low (0.2 mg Zn kg^{-1}) and high (5 mg Zn kg^{-1}), on mycorrhiza infection rates of 66-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants harvested at early flowering stage.

In case of low P supply, hyphae of mycorrhiza surrounded plant roots intensively, whereas the mycorrhizae growth negatively affected by increasing P supply and completely arrested at high P until a point where hyphae were no longer visible (Fig 1.3). Figure 1.3 represents the conditions at low Zn treatment (at high Zn supply results were very similar).

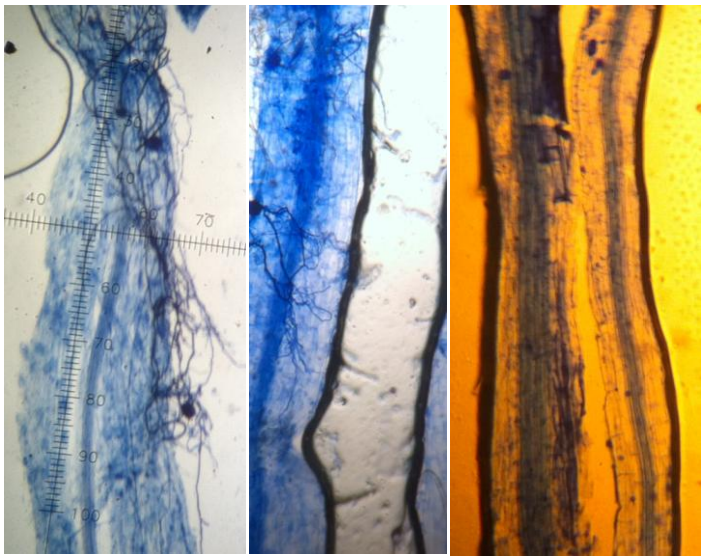


Fig 1.3: Mycorrhizal infection of the roots of bread wheat (*Triticum aestivum* cv. Adana99) plants after 66 days of growth at early flowering stage and low Zn supply. Plants were grown with low (left), adequate (middle) and high (right) P treatments.

1.4. Discussion

In well agreement with the previous results (Loneragan et al 1982, Cakmak and Marschner 1986; Nichols et al. 2012) increases in P supply induced Zn deficiency and reduced growth of plants under low Zn supply (Table 1.1; Fig. 1.1). When Zn was sufficiently high in growth medium, high P remained ineffective on growth of plants. The decreases in growth of plants with high P supply were more pronounced in case of grain yield. High P treatments reduced grain yield more severely than the vegetative growth which indicate that generative growth is more susceptible to low Zn conditions than the vegetative growth. Similar results were also found in wheat grown under field conditions (Yilmaz et al., 1997). Zinc is known as a micronutrient which has large positive impacts on pollination and pollen viability. Under low Zn supply, pollination process is severely depressed (Marschner 2012). Consequently, generative growth is more affected from Zn deficiency than the vegetative growth. It is therefore important to ensure a better Zn nutrition of plants during the reproductive growth stage, for example by spraying foliar Zn fertilizers.

There are controversial results on the impact of increasing P supply on shoot and grain Zn concentrations in literature. In the present study, high P supply significantly reduced Zn concentrations of shoots and grains (Tables 1.2 and 1.4). These results are in good agreement

with the results of Ryan et al (2008) and Zhang et al (2012); but in disagreement with the results of Lonereagan et al. (1982), Cakmak and Marschner (1986, 1987), Nichols et al (2012). It is important to highlight that the inhibitory impact of high P on shoot or grain Zn was found in experiments conducted on soils while in the studies realized in nutrient solution high P had no influence on shoot Zn. Based on these results it can be suggested that there is most probably no direct interaction between P and Zn such as precipitation of Zn with P in growth medium. As discussed in the Chapter 3, even high P treatments stimulated root Zn uptake. It is very obvious to suggest that any precipitation or complexation between P and Zn can be excluded as a factor to be responsible for the decline in grain or shoot concentrations of Zn upon high P applications.

It seems very likely that high P causes other conditions for soil-grown plants which depresses root Zn uptake and thus shoot Zn concentrations of plants. Mycorrhizae is a well-known fungi contributing greatly to root Zn uptake (Kothari et al., 1991; Li et al., 2003; Marschner, 2012), and its positive impact on root Zn uptake is very much dependent on P status of the soils. As shown in those studies, increases in P application, mycorrhizal infection of plants is reduced and thus, Zn uptake of plants is also declined. It is very obvious that high P reduces root Zn uptake and shoot Zn concentration of plants by depressing the mycorrhizal infection of roots. In good agreement with this suggestion, it has been found in the present study that increases in P supply impaired very significantly mycorrhizal infection of roots (Fig. 1.2). Probably, due to this effect of high P on mycorrhizae, plants with high P had less Zn. This reducing effect of high P on shoot or grain Zn concentrations is very specific for Zn, and could not be found in case of other nutrients analyzed. Since mycorrhizae have also very specific impact on Zn uptake and accumulation of plants (Marschner 2012) it can be emphasized that decreases in shoot or grain Zn by increasing P supply is most probably caused by depressed mycorrhizal infection of roots.

Alternatively, a reduction in root Zn uptake and shoot or grain Zn accumulation can be a result of reduced root growth by increasing P supply (Marschner, 2012). But, this possibility can be excluded because, as highlighted above, decreases in shoot or grain Zn by high P is very specific for Zn and could not be found in case of other nutrients. Zhang et al (2012) has also showed that decreases in grain Zn by increasing P supply was found only in case of Zn; not in case of Fe, Mn or Cu.

The possibility that high P causes accumulation of Zn in roots and impairs the root-to-shoot transport of Zn is very reasonable that was not studied in this chapter. There are

published results in literature showing that root Zn concentration is markedly increasing by increasing P supply (Younghdahl et al., 1977; Nichols et al., 2012). This issue has been discussed in more detail in the Chapter 3.

1.5. Conclusions

In conclusion it can be suggested that mycorrhizae is an important player in explanation of PxZn interaction of plants grown in soils. There is a common discussion in literature on whether Zn is precipitated with P in growth medium or in Zn-P granular fertilizers. Based on the results here and also in the Chapter 3 it can be suggested that any P-Zn precipitation in growth medium or fertilizer granules is most probably unlikely.

CHAPTER 2

INCREASING PHOSPHORUS APPLICATION CAUSES REDUCTION IN ZINC CONCENTRATIONS OF PLANTS GROWN IN NATIVE SOIL BUT NOT IN PLANTS GROWN IN STERILIZED SOIL

2.1. Introduction

In most cases, root Zn uptake is limited under high P availability in growth medium while root P uptake is increased in the presence of low Zn, which points out existence of an antagonism between Zn and P (Mousavi, 2011). Soil experiments seemed to be distinctive from hydroponic experiments where Zn levels of plants did not change with respect to increasing P applications (Cakmak and Marschner, 1987; Nichols et al., 2012). Since mycorrhiza do not exist in solution culture and its colonization is dramatically reduced with P availability (Li et al., 2006), it appears that growth medium of the PxZn studies is an important parameter to be taken into consideration when the nature of the PxZn antagonism is investigated.

In the Chapter 1, it was demonstrated that activity of mycorrhiza exist in the experimental soil and its infection rates were strongly depended on P fertilization. The results of the Chapter 1 related to mycorrhiza were in good agreement with the results available in the literature (Li et al., 2003; Marschner, 2012). As expected, high P availability reduced mycorrhiza infection of roots, and probably through this reduction in mycorrhizae activity plant Zn concentrations were also reduced as shown in the Chapter-I. Mycorrhizae are known to exert a positive influence on the root uptake of Zn from the soil by extending its hyphae deeper in bulk soil where roots normally can't reach (Smith and Read, 2008). Therefore, at high P treatments, mycorrhiza activity is very low and cannot contribute to root uptake of Zn, leading to a lower plant Zn concentration as shown in previous studies (Kothari et al., 1991; Ryan et al., 2008).

In order to investigate the impact of mycorrhizae on the P and Zn interaction, additional experiments were conducted by using sterilized (autoclaved) soils. As in the Chapter-I, bread wheat plants were grown in soil with two different Zn and three different P concentrations by using autoclaved soil and native soil. Main question of this chapter was how shoot are and grain concentrations are affected with respect to increasing P treatments in a soil that was either native or sterilized to avoid impact of mycorrhizae on PxZn interaction.

2.2. Materials and Methods

The experiment in this chapter was conducted to understand role of mycorrhiza in high P-induced Zn deficiency and reductions in Zn uptake in wheat, and designed as a factorial experiment with 5 independent replicates.

Before setting up the experiment, part of the experimental soil was autoclaved at 121^{o}C for 2 hours in order to eliminate activity of microorganisms as much as possible, and used for the half of the pots.

Each plastic pot was filled with 3.1 kg of soil and then supplied with 300 mg N in the form of $Ca(NO_3)_2.4H_2O$ with 25 mg S in the form of K_2SO_4 per kg of soil. Three different rates of P were used as following: 15 mg kg^{-1} (low), 60 mg kg^{-1} (adequate) and 180 mg kg^{-1} (high) in the form $Ca(H_2PO_4)_2$ and two different Zn concentrations were used: 0.2 mg kg^{-1} and 5 mg kg^{-1} in the form of $ZnSO_4{\cdot}7H_2O$. All fertilizers added into the pots were homogenously incorporated into the soil.

Twelve seeds were sown in each pot and upon emergence the seedling numbers were thinned down to five. Two plants were harvested after 68 days at anthesis. The remaining three plants were harvested at maturity. On the 58th day every pot was supplied with 100 mg kg^{-1} of additional N in the form of $Ca(NO_3)_2.4H_2O$ to avoid any risk with N deficiency. The pots were watered daily with deionized water. Harvested plants were dried, weighed and analyzed for element composition as indicated in “General Materials and Methods”.

2.3. Results

Both P and Zn treatments had a significant impact on the shoot dry matter production of wheat plants. Overall, increasing P fertilization improved shoot dry matter production of plants at both low and high Zn supply in each soil used indicating that plants under low P supply was severely affected from P deficiency (Table 2.1). When the soil was sterilized, plant dry matter production tended to increase in most of the treatments which might be related to elimination of some soil-borne pathogens in growth medium and/or increased pool of available mineral nutrients due to their mobilization/mineralization after sterilization of soils.

Table 2.1: Shoot dry matter production of 68 day-old bread wheat (*Triticum aestivum* cv. Adana99), grown at low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P, with two different Zn treatments as low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}), and two different soil treatments (non-sterilized and sterilized).

Zn Treatment	Soil Treatment	P Treatment	Shoot DW (g $plant^{-1}$)
Low	Non-Sterilized	Low	3.5 ± 0.5
		Adequate	4.3 ± 0.6
		High	4.2 ± 0.4
	Sterilized	Low	2.9 ± 0.6
		Adequate	5.3 ± 1.0
		High	4.4 ± 0.6
Adequate	Non-Sterilized	Low	3.6 ± 0.2
		Adequate	5.3 ± 1.1
		High	5.8 ± 0.2
	Sterilized	Low	3.8 ± 0.8
		Adequate	5.5 ± 0.3
		High	6.6 ± 0.6

Shoot DW $HSD_{0.05}$ (Zn; Soil; P; ZnxSoil; ZnxP; SoilxP; ZnxSoilxP): (0.3; *n.s*; 0.5; *n.s*; 0.9; *n.s*; *n.s*)

When plants were grown under low Zn and exposed to high P there was a very clear depression in growth of plants. Low Zn plants under high P treatment exhibited necrotic spots on leaves, mainly on middle-aged leaves, and developed a stunting growth (Figs. 2.1

and 2.2). Zinc deficiency symptoms described became more severe under high P treatment over time (Figs. 2.1 and 2.2). As a result of those leaf symptoms, plant dry matter production was severely reduced in low Zn plants at high P supply (Table 2.1). When Zn supply was adequate, high P did not cause any leaf symptoms and plants looked healthy. Sterilization did not affect too much expression of the Zn deficiency symptoms, but plants had higher dry matter production as discussed above.

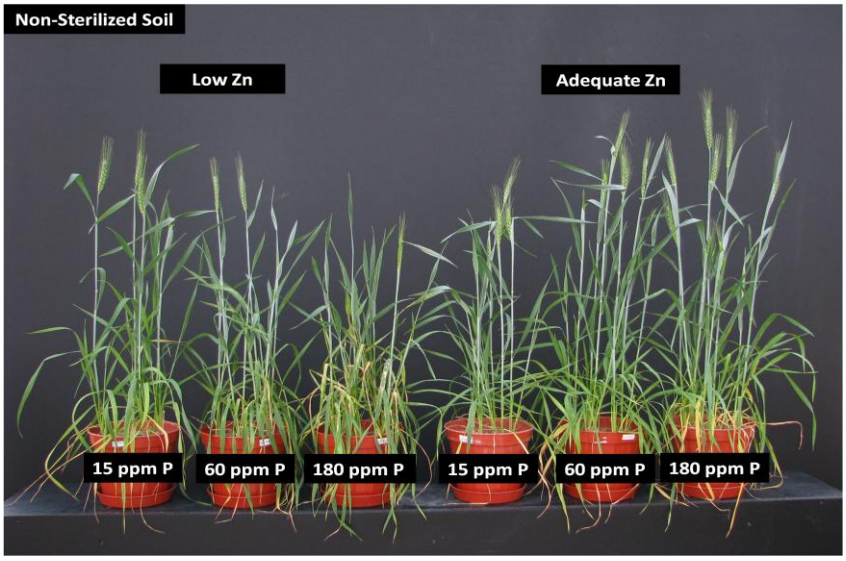


Fig 2.1: Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P applications on growth of 63 days-old bread wheat (*Triticum aestivum* cv. Adana99) plants with low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}) Zn supply in the non-sterilized (native) under greenhouse conditions.

On the other hand, P deficiency symptoms (e.g. reduction in shoot growth and dark leaf color) appeared noticeably earlier in plants subjected to low P supply, especially in case of adequate Zn supply (Fig 2.1; Fig 2.2). The negative impact of low P supply was also visible in other results of this experiment such as low shoot P concentrations and grain yields as presented below.

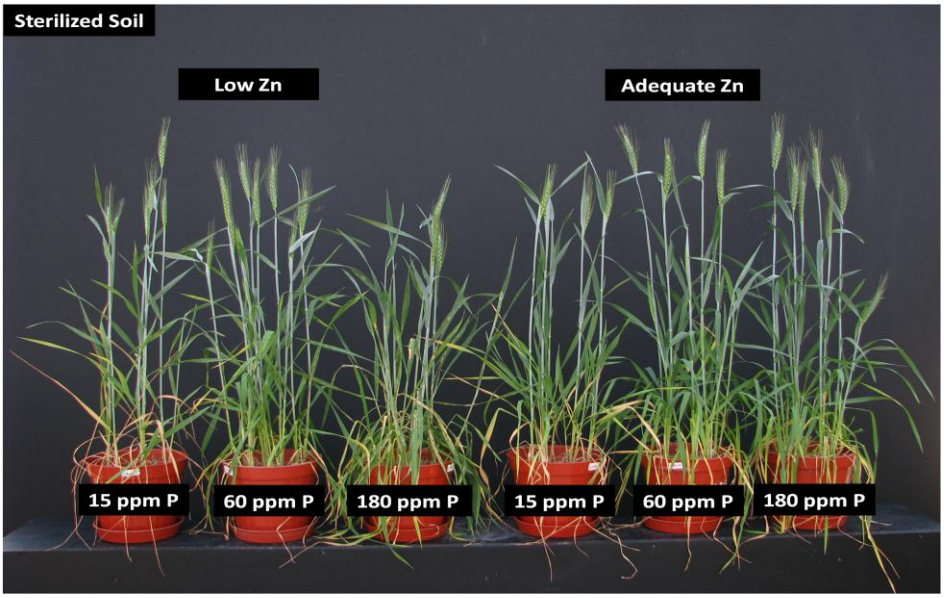


Fig.2.2: Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P applications on growth of 63 days-old bread wheat (*Triticum aestivum* cv. Adana99) plants with low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}) Zn supply in the sterilized (autoclaved) under greenhouse conditions.

As shown in Table 2.2, plants in non-sterilized soil exhibited a declining trend in shoot Zn concentration with respect to increasing P supply. The fall in shoot Zn concentrations by high P reaches up to 47% at low Zn and 73% at high Zn treatments. On the other hand, when a sterilized soil was used, shoot Zn concentrations showed a different picture (Table 2.2). There was either no or only very slight change in shoot Zn concentrations by increasing P supply. Changes in shoot Zn concentrations with respect to P was statistically insignificant in sterilized soil.

In both non-autoclaved and autoclaved soils, shoot P concentrations of plants rise with increasing P supply (Table 2.2). The increases in shoot P concentrations looked very similar in all groups. At low P conditions, plants grown in non-sterilized soil had significantly more P than the plants grown in sterilized soil.

Table 2.2: Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P applications on shoot Zn and P concentrations of 68 days-old bread wheat (*Triticum aestivum* cv. Adana99) plants with low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}) Zn supply in the sterilized (autoclaved) and non-sterilized (native) soil under greenhouse conditions.

Zn Treatment.	Soil Treatment	P Treatment	Zn Concentration (mg kg^{-1})	P Concentration (% dry wt)
Low	Non-Sterilized	Low	17 ± 2	0.14 ± 0.02
		Adequate	8 ± 0	0.21 ± 0.01
		High	9 ± 2	0.26 ± 0.04
	Sterilized	Low	6 ± 1	0.06 ± 0.00
		Adequate	7 ± 1	0.13 ± 0.01
		High	7 ± 1	0.25 ± 0.02
Adequate	Non-Sterilized	Low	60 ± 9	0.12 ± 0.00
		Adequate	24 ± 2	0.16 ± 0.02
		High	16 ± 2	0.21 ± 0.03
	Sterilized	Low	18 ± 2	0.07 ± 0.01
		Adequate	17 ± 1	0.12 ± 0.01
		High	17 ± 1	0.20 ± 0.01

Zn Conc. $HSD_{0.05}$ (Zn; Soil; P; ZnxSoil; ZnxP; SoilxP; ZnxSoilxP): (2; 2; 2; 3; 4; 4; 7)

P Conc. $HSD_{0.05}$ (Zn; Soil; P; ZnxSoil; ZnxP; SoilxP; ZnxSoilxP): (0.01; 0.01; 0.01; *n.s*; 0.02; 0.02; *n.s*)

Sterilization process seemed to make plants healthier; but in case of low Zn supply, plants suffered severely from Zn deficiency due to much lower shoot Zn concentrations (Table 2.2).

The grain yield per plant did not change much in low Zn and non-autoclaved soil with respect to P treatment (Table 2.3). On the other hand, in low Zn and sterilized soil situation, low P fertilization reduced grain yield significantly (Table 2.3). In the sterilized soil, grain production was at least doubled at adequate or high P treatments when compared to low P in sterilized soil. In case of adequate Zn supply, grain yield of low P plants increased by adequate P supply in both soils, and even further increased in sterilized soil when P is applied at high rate (Table 2.3). High P treatment promoted grain yield about 32% in non-sterilized

soil and about 160% in sterilized soil groups with adequate Zn supply. In nearly all treatments, increasing P supply gradually improved straw dry matter production (Table 2.3). Interestingly, at a given P treatment, straw dry matter production values looked very similar between all other treatments.

Table 2.3: Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P applications on grain yield and straw dry matter production of bread wheat (*Triticum aestivum* cv. Adana99) plants grown until grain maturation with low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}) Zn supply in the sterilized (autoclaved) and non-sterilized (native) soil under greenhouse conditions.

Zn Treatment	Soil Treatment	P Treatment	Grain Yield (g $plant^{-1}$)	Straw DW (g $plant^{-1}$)
Low	Non-Sterilized	Low	2.5 ± 0.3	2.2 ± 0.3
		Adequate	2.8 ± 0.3	3.1 ± 0.5
		High	2.5 ± 0.5	3.0 ± 0.2
	Sterilized	Low	1.2 ± 0.1	2.1 ± 0.1
		Adequate	2.5 ± 0.2	2.9 ± 0.3
		High	2.8 ± 0.3	3.4 ± 0.3
Adequate	Non-Sterilized	Low	2.5 ± 0.2	2.1 ± 0.3
		Adequate	3.3 ± 0.4	2.8 ± 0.3
		High	3.3 ± 0.4	2.9 ± 0.3
	Sterilized	Low	1.4 ± 0.3	2.0 ± 0.4
		Adequate	3.5 ± 0.2	3.3 ± 0.2
		High	3.7 ± 0.2	3.2 ± 0.3

Grain Yield $HSD_{0.05}$ (Zn; Soil; P; ZnxSoil; ZnxP; SoilxP; ZnxSoilxP): (0.1; 0.1; 0.2; *n.s*; 0.4; 0.4; *n.s*)
Straw DW $HSD_{0.05}$ (Zn; Soil; P; ZnxSoil; ZnxP; SoilxP; ZnxSoilxP): (*n.s*; *n.s*; 0.2; *n.s*; *n.s*; *n.s*; *n.s*)

Grain Zn concentrations responded to increasing P supply with very obvious decreases at each Zn treatment, particularly in non-sterilized soil (Table 2.4). There was up to 3-fold and 2-fold decreases in grain Zn under low and adequate Zn supply in non-sterilized soil,

respectively. In case of sterilized soil, the decreases in grain Zn with high P were very slight (Table 2.4).

As expected, high Zn treatment enhanced grain Zn concentrations very significantly at each other treatment (Table 2.4). Like in shoot samples, soil sterilization very significantly depressed grain Zn concentrations of plants, respectively. Phosphorus concentrations of grains were distinctly increased by increasing P supply under both Zn treatments and soil sterilization treatments (Table 2.4). In case of soil sterilization, grain P concentrations were much lower than the grain samples without soil sterilization, especially in case of low and adequate P supply (Table 2.4), indicating a depressing effect of soil sterilization on root P uptake like in case of Zn.

Table 2.4: Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P applications on grain Zn and P concentrations of bread wheat (*Triticum aestivum* cv. Adana99) plants grown until grain maturation with low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}) Zn supply in the sterilized (autoclaved) and non-sterilized (native) soil under greenhouse conditions

Zn Treatment	Soil Treatment	P Treatment	Zn Concentration (mg kg^{-1})	P Concentration (% dry wt)
Low	Non-Sterilized	Low	32 ± 5	0.26 ± 0.03
		Adequate	18 ± 1	0.47 ± 0.01
		High	11 ± 1	0.51 ± 0.03
	Sterilized	Low	10 ± 1	0.18 ± 0.02
		Adequate	8 ± 0	0.28 ± 0.02
		High	8 ± 1	0.47 ± 0.01
Adequate	Non-Sterilized	Low	67 ± 3	0.25 ± 0.01
		Adequate	54 ± 4	0.43 ± 0.02
		High	30 ± 4	0.47 ± 0.03
	Sterilized	Low	33 ± 4	0.18 ± 0.02
		Adequate	25 ± 2	0.24 ± 0.02
		High	27 ± 2	0.41 ± 0.01

Zn Conc. $HSD_{0.05}$ (Zn; Soil; P; ZnxSoil; ZnxP; SoilxP; ZnxSoilxP): (1; 1; 2; 3; 4; 4; 6);

P Conc. $HSD_{0.05}$ (Zn; Soil; P; ZnxSoil; ZnxP; SoilxP; ZnxSoilxP): (0.01; 0.01; 0.01; *n.s*; 0.03; 0.03; *n.s*)

In contrast to Zn, grain K and Mg concentrations were increased by increasing P application under different Zn treatments, and this result was not also affected from the soil sterilization (Table 2.5). In case of grain Ca, the changes were very slight upon the treatments applied. Similarly also grain concentrations of Fe and Mn were either not changed or even increased by increasing P applications (Table 2.6). In the non-sterilized soil, increase in P supply tended to decrease grain Cu while in sterilized soil there was a slight increase in grain Cu with high P (Table 2.6).

Table 2.5: Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P applications on grain K, Mg and Ca concentrations of bread wheat (*Triticum aestivum* cv. Adana99) plants grown until grain maturation with low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}) Zn supply in the sterilized (autoclaved) and non-sterilized (native) soil under greenhouse conditions.

Zn Treatment	Soil Treatment	P Treatment	K (%)	Mg (%)	Ca (%)
Low	Non-Sterilized	Low	0.34 ± 0.01	0.13 ± 0.01	0.04 ± 0.00
		Adequate	0.46 ± 0.03	0.17 ± 0.00	0.04 ± 0.00
		High	0.48 ± 0.06	0.18 ± 0.01	0.04 ± 0.00
	Sterilized	Low	0.33 ± 0.02	0.10 ± 0.01	0.04 ± 0.00
		Adequate	0.36 ± 0.02	0.13 ± 0.01	0.03 ± 0.00
		High	0.44 ± 0.02	0.17 ± 0.00	0.03 ± 0.00
Adequate	Non-Sterilized	Low	0.32 ± 0.01	0.12 ± 0.00	0.04 ± 0.00
		Adequate	0.42 ± 0.03	0.16 ± 0.00	0.04 ± 0.00
		High	0.44 ± 0.02	0.16 ± 0.01	0.04 ± 0.00
	Sterilized	Low	0.31 ± 0.01	0.11 ± 0.00	0.04 ± 0.00
		Adequate	0.33 ± 0.02	0.13 ± 0.01	0.03 ± 0.00
		High	0.38 ± 0.02	0.15 ± 0.01	0.04 ± 0.00

K Conc. $HSD_{0.05}$ (Zn; Soil; P; ZnxSoil; ZnxP; SoilxP; ZnxSoilxP): (0.01; 0.01; 0.02; *n.s*; 0.03; *n.s*; *n.s*)

Mg Conc. $HSD_{0.05}$ (Zn; Soil; P; ZnxSoil; ZnxP; SoilxP; ZnxSoilxP): (0.01; 0.01; 0.01; *n.s*; 0.01; 0.01; *n.s*)

Ca Conc. $HSD_{0.05}$ (Zn; Soil; P; ZnxSoil; ZnxP; SoilxP; ZnxSoilxP): (0.01; 0.01; 0.02; *n.s*; 0.01; 0.03; *n.s*)

Table 2.6: Effect of low (15 mg P kg^{-1}), adequate (60 mg P kg^{-1}) and high (180 mg P kg^{-1}) P applications on grain Fe, Cu and Mn concentrations of bread wheat (*Triticum aestivum* cv. Adana99) plants grown until grain maturation with low (0.2 mg Zn kg^{-1}) and adequate (5 mg Zn kg^{-1}) Zn supply in the sterilized (autoclaved) and non-sterilized (native) soil under greenhouse conditions.

Zn Treatment	Soil Treatment	P Treatment	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})
Low	Non-Sterilized	Low	25 ± 2	7.1 ± 0.7	30 ± 2
		Adequate	30 ± 2	6.4 ± 0.2	43 ± 2
		High	31 ± 2	6.4 ± 0.2	45 ± 5
	Sterilized	Low	28 ± 2	4.8 ± 0.1	55 ± 4
		Adequate	29 ± 2	5.8 ± 0.3	61 ± 9
		High	29 ± 2	6.7 ± 0.4	83 ± 6
Adequate	Non-Sterilized	Low	26 ± 2	8.2 ± 0.3	30 ± 3
		Adequate	26 ± 2	7.0 ± 0.5	43 ± 3
		High	28 ± 1	5.0 ± 0.2	44 ± 2
	Sterilized	Low	30 ± 4	5.1 ± 0.6	65 ± 8
		Adequate	29 ± 3	5.5 ± 0.4	55 ± 6
		High	35 ± 3	5.5 ± 0.4	72 ± 6

Fe Conc. $HSD_{0.05}$ (Zn; Soil; P; ZnxSoil; ZnxP; SoilxP; ZnxSoilxP): (*n.s*; 1; 2; 2; 3; *n.s*; 5)

Cu Conc. $HSD_{0.05}$ (Zn; Soil; P; ZnxSoil; ZnxP; SoilxP; ZnxSoilxP): (*n.s*; 1; 1; 1; 1; 1; *n.s*)

Mn Conc. $HSD_{0.05}$ (Zn; Soil; P; ZnxSoil; ZnxP; SoilxP; ZnxSoilxP): (*n.s*; 3; 4; *n.s*; 7; 7; 12)

2.4. Discussion

Plants severely suffered from Zn deficiency at low Zn and high P supply. Increasing rate of P treatment under low Zn promoted expression of Zn deficiency stress by reducing shoot Zn concentrations. With sterilized soil, impact of high P on Zn concentrations was more distinct (Table 2.1). Despite much lower shoot Zn concentrations of plants on sterilized soil, shoot growth was not more reduced at high P treatments when compared to the non-sterilized soil under low Zn treatment. This unexpected result could be related to better growth of plants on soils with sterilization (Smith and Smith, 1981).

When soil is sterilized, then the contribution of mycorrhizae to P and Zn uptake is limited which may induce more severe deficiency situation with Zn and P. Mycorrhiza is able extends its hyphae out of rhizosphere and use also P and Zn sources out of the rhizosphere soil (Tinker et al., 1992; Smith and Read, 2008). This ability of mycorrhizae is lost when soil is sterilized which consequently contributes to more severe P and Zn deficiency in plants. This adverse effect of soil sterilization on shoot growth was also found on grain yield results (Table 2.3). In case of low P supply, grain yield of plants was reduced markedly when soils were sterilized. Accordingly, grain concentrations of Zn and P were significantly depressed by soil sterilization (Table 2.4).

In a field experiment, Singh et al. (1986) showed a very clear drop in plant Zn concentrations upon P treatment in the presence of mycorrhiza, and this depressive effect of high P on Zn concentrations of plants was ascribed to elimination of positive effect of mycorrhizae on root Zn uptake.. The results presented here are in well agreement with the results reported by Singh et al (1986). It has been well-documented that mycorrhiza also greatly contributes to P uptake of plants, especially in soils with low P supply (or availability) (Li et al., 2006; Smith and Read, 2008). As demonstrated in this study, elimination of mycorrhizae fungi in growth medium through soil sterilization caused significant decreases in shoot and grain concentrations of P, indicating importance of mycorrhizae in root P uptake of plants. These results are in well accordance with the results published previously (Kothari et al., 1991; Li et al., 2006; Marschner, 2012).

As was the case with P, also shoot and grain concentrations of Zn were severely depressed with soil sterilization under both Zn treatments which also highlights importance of mycorrhizae in Zn nutrition of plants (Table 2.4). Interestingly, marked decreases in shoot or grain Zn concentrations by increasing P supply was not found in the same magnitude in the soil which was sterilized (Table 2.4). For example, at low Zn supply and in non-sterilized soil, an increase in P supply from adequate to high rate reduced grain Zn concentration from 18 mg kg^{-1} to 11 mg kg^{-1} while in sterilized soil grain Zn concentration was not affected from different P applications. Similarly in soil with adequate Zn supply and non-sterilized, there was a substantial decrease in grain Zn with high P supply (e.g., from 54 to 30 mg kg^{-1}); but in sterilized soil grain Zn was even slightly increased by high P (Table 2.4). These results suggests a highly significant role of mycorrhizae in shoot and grain Zn concentration and indicate minor role of a possible P-Zn precipitation in rhizosphere as a reason for high P-reduced shoot Zn concentrations.

In studies with bean plants, Gianquinto et al. (2000) explained the reduction of Zn concentrations upon high P treatments by a dilution effect (e.g., dilution of tissue Zn by enhanced growth at high P). This dilution effect of high P on plant Zn might be also important in this study; but it cannot be a major and relevant factor because the decline in plant Zn by high P seems to be very specific for Zn and could not be found in case of other nutrients. In addition, the depressive effect of high P on grain Zn was either very slight or disappeared when the soils were sterilized (Table 2.2; Table 2.4).

In contrast to grain Zn, Mg concentrations of grain showed clear increases by increasing P supply (Table 2.5). A similar effect of P on grain Mg was also demonstrated in a previous study on maize, rice wheat, and common bean plants (Fageria et al., 1995). It is well-known that increases in grain P concentrations are closely associated with increase in grain phytate (Lu et al., 2011). High phytate in seeds can create a sink for cations due to its high binding capacity for cations such as Mg, K and Ca (Raboy, 1997; Welch and Graham, 2004). Due to this effect, an increase in P and thus phytate in seeds were possibly associated with increases in K and Mg (Table 2.5).

Soil sterilization caused also marked increases in grain (Table 2.6) and shoot (data not shown) concentrations of Mn. It is well known that during the autoclave process, when microorganisms in the soil were killed, there is a very high Mn-mineralization which causes marked increases in soil Mn availability and thus high potential for enhanced root Mn uptake (Kothari et al., 1991; Marschner, 2012). Accordingly, in the present study, soil sterilization increased DTPA-extractable Mn concentration of the experimental soil nearly 8-fold (from 0.99 to 7.44 mg kg^{-1} soil; data not shown). There are also additional explanations in literature regarding why mycorrhizal plants tend to have lower Mn in tissue. Colonization of roots with mycorrhizae fungus greatly affects soil microbial community resulting with a lower density in the population of Mn reducing bacteria and thus less available Mn in rhizosphere for root uptake (Arines et al., 1989; Kothari et al., 1991).

2.5. Conclusions

The decline in grain and shoot Zn concentrations with respect to increasing P treatments was found to be substantial which then stimulated expression of Zn deficiency in plants when Zn supply was low in soils. However, when soil was sterilized, this depressive effect of high P on plant Zn was significantly diminished or even disappeared indicating that mycorrhizae is a very critical player in occurrence of interaction between P and Zn. Thus, P induced Zn deficiency cannot be explained by any precipitation of P with Zn in soil. This issue is being discussed in more detail in the Chapter-III.

CHAPTER 3

ROOT ZINC UPTAKE IS PROMOTED IN PLANTS GROWN WITH HIGH PHOSPHORUS CONCENTRATION IN NUTRIENT SOLUTION

3.1. Introduction

The antagonistic interaction between P and Zn was mainly reported in soil trials. In these experiments, Zn levels of plant tissues were dramatically reduced by increasing phosphorus availability (Singh et al., 1988; Zhu et al., 2001; Li et al., 2003). In contrast, tissue Zn levels were not affected by P supply in nutrient solution studies (Cakmak and Marschner 1987; Nichols et al. 2012). Whether the growth medium is soil or nutrient solution appears to be the reason behind these contradictory reports. One of the most important differences between these growth conditions is the absence of mycorrhiza in nutrient culture.

The results presented in chapter 2 did not support the idea that P precipitates with Zn. Moreover, dilution of Zn in shoot tissues due to increased shoot-to-root ration at high P supply was probably not the main factor behind the interaction between P and Zn. The results pointed out to the tremendous impact of mycorrhiza under limited nutrient supply. Eliminating mycorrhiza from the soil resulted in totally different outcomes. Mycorrhizal fungi are absent under solution culture conditions.

In order to understand the interaction between P and Zn in plant growth, investigating the response of plants to varied P and Zn supply in hydroponics could provide important clues. Nutrient solution cultures are quiet distinct from complex soil systems where plant growth is affected by soil microorganisms, rhizosphere phenomena, mineral interactions etc. In hydroponic cultures, there is far less complexity, and as a result, it is possible to observe the response of plants to P and Zn excluding factors like mycorrhiza.

Impaired root-to-shoot translocation of Zn under high P conditions is one of the explanations reported for the interaction of P with Zn (Khanif and Saleem, 2013). It was also proposed that P can induce Zn deficiency by reducing the water solubility of Zn (Alloway, 2004).

In this chapter, the following questions were addressed:

i) How do the shoot and root mineral concentrations and the plant growth respond to varied P supply under low or high Zn conditions in solution culture?

ii) How is the translocation of Zn from root to shoot affected by P and Zn treatments?

iii) How Zn uptake rate was affected by different P and Zn levels in the absence of mycorrhiza?

3.2. Materials and Methods

Two separate hydroponic experiments were carried out. The first one was conducted under greenhouse and the second one was conducted under growth chamber conditions as described below:

3.2.1. First Experiment

The first experiment was carried out to observe the effects of different P and Zn applications on the plant growth and mineral concentrations of the shoot and root. It was conducted under greenhouse conditions.

Plants were supplied with 3 different P concentrations (20 μM, 100 μM and 500 μM) in the form of $Ca(H_2PO_4)_2$ and 3 different Zn concentrations (0.01 μM, 0.1 μM and 1 μM) in the form of $ZnSO_4 \cdot 7H_2O$. Low P and adequate P pots were supplemented with 480 μM and 400 μM $CaSO_4.2H_2O$, respectively.

After 34 days of growth, shoots and roots were harvested and dried.

This work was designed as a factorial experiment containing 4 replicates. Each pot contained 20 plants.

3.2.2. Second Experiment

The second experiment was carried out to investigate the Zn uptake rate at different P and Zn supply levels. It was carried out under growth chamber conditions. The experiment had a full factorial design with 4 replicates. Each pot contained 8 plants.

Nutrient solutions were supplied with 3 different P concentrations (20 μM, 100 μM and 500 μM) in the form of $Ca(H_2PO_4)_2$ and 2 different Zn concentrations (0.01 μM and 1 μM) in the form of $ZnSO_4 \cdot 7H_2O$. Low P and adequate P pots were supplemented with 480 μM and 400 μM $CaSO_4.2H_2O$ respectively.

After 19 days of growth, 2 plants were transferred to Erlenmeyer flasks containing nutrient solution diluted 1:10 and kept there for 30 minutes. They were then transferred to another group of Erlenmeyer flasks containing concentrated nutrient solution containing 2 μM Zn but no other micronutrients. 3 ml samples were taken from the flasks every hour for 7 hours in order to follow the depletion of Zn from the solution culture. The first samples were taken right before the transfer of plants. Upon terminating the uptake study, plants were once again transferred to diluted (1:10) complete nutrient solution and kept there for 30 min. Then, they were grown for another day under standard conditions. All plants were then harvested.

3.2.3. Root Cleaning Procedure

Roots were first washed in deionized water, then in 1 mM $CaCl_2$ for 30 min, then in 1 mM EDTA for 30 min and finally again in deionized water.

3.3. Results

In the first experiment conducted under greenhouse conditions, the shoot dry weight responded positively to increasing P and Zn applications (Table 3.1). The lowest value was obtained at low Zn and low P supply. Under low Zn conditions, adequate P supply led to the highest biomass. High P impaired plant growth at low Zn. However, at medium and high Zn, high P supply had a significant positive impact on plant growth. Improving Zn supply from

low to medium boosted the shoot dry matter production, but increasing it from medium to high did not have an additional effect.

The root biomass was also very much affected by the P supply level (Table 3.1). The low P treatment encouraged plants to produce more root at all Zn treatments. Moreover, increasing Zn application remarkably reduced root growth. When the root-to-shoot ratio was taken into account, the effect of P was more marked than the effect of Zn. At low P and Zn, the highest root-to-shoot ratio was observed. The ratio was significantly reduced at adequate and high P.

Table 3.1: Shoot and root dry matter production of 34-day-old bread wheat (*Triticum aestivum* cv. Adana99), grown at low (20 µM P), adequate (100 µM P) and high (500 µM P) P, with three different Zn treatments as low (0.01 µM Zn), medium (0.1 µM Zn) and high (1 µM Zn).

Zn Treat.	P Treat.	Shoot (g plant^{-1})	Root (g plant^{-1})	Root/Shoot (%)
Low	Low	0.15 ± 0.00	0.11 ± 0.01	78 ± 1
	Adequate	0.21 ± 0.03	0.08 ± 0.01	35 ± 4
	High	0.17 ± 0.02	0.09 ± 0.02	45 ± 9
Medium	Low	0.21 ± 0.01	0.09 ± 0.01	45 ± 1
	Adequate	0.28 ± 0.01	0.07 ± 0.01	26 ± 2
	High	0.28 ± 0.01	0.06 ± 0.00	23 ± 1
High	Low	0.22 ± 0.01	0.10 ± 0.01	48 ± 2
	Adequate	0.27 ± 0.03	0.06 ± 0.02	26 ± 2
	High	0.30 ± 0.01	0.07 ± 0.01	23 ± 4

Shoot DW $HSD_{0.05}$ (Zn; P; ZnxP): (0.02; 0.02; *n.s*)

Root DW $HSD_{0.05}$ (Zn; P; ZnxP): (0.01; 0.01; 0.04)

Root/Shoot Ratio $HSD_{0.05}$ (Zn; P; ZnxP): (4; 4; 9)

The adverse effect of higher P supply at low Zn case can be observed in Fig 3.1. At low Zn, low P plants exhibited severe Zn deficiency symptoms. Increasing the P supply to the medium level slightly improved the plant vigor but Zn deficiency symptoms became more widespread. At high P, tissue necrosis due to Zn deficiency

was extremely severe. In contrast, higher levels of P enhanced the shoot biomass production at high Zn. All Zn deficiency symptoms disappeared under high Zn conditions.

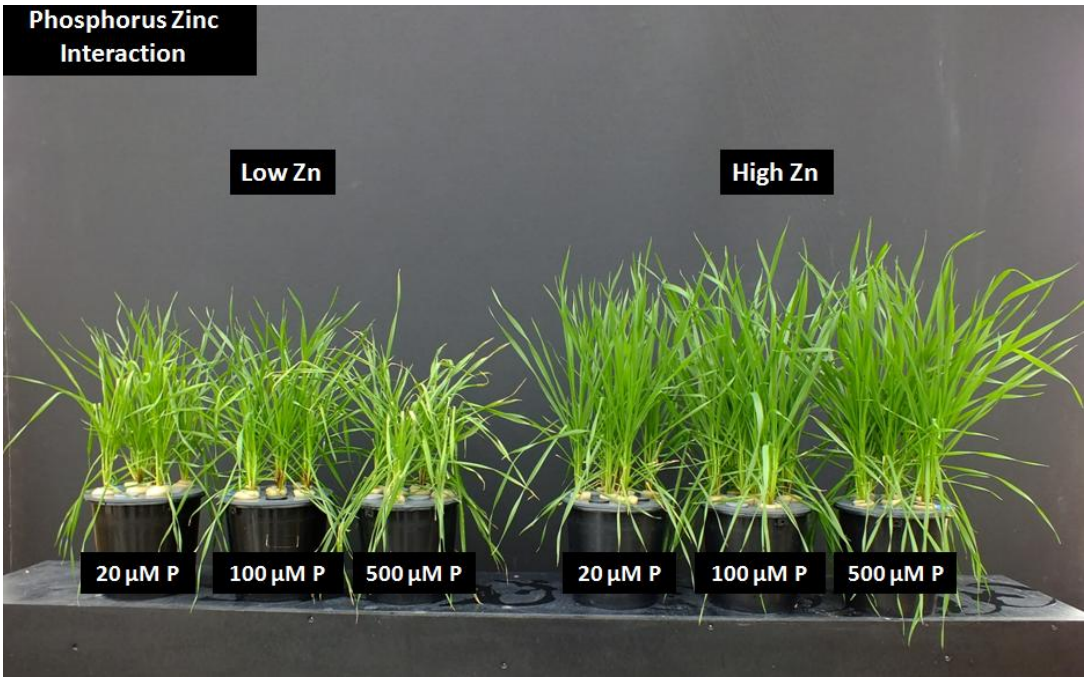


Fig.3.1: Effect of low (20 µM P), adequate (100 µM P) and high (500 µM P) P, with two different Zn treatments as low (0.01 µM Zn) and high (1 µM Zn), on growth of 34-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants.

The shoot Zn concentrations were almost quadrupled when the Zn supply was increased from low to medium, and then again doubled when it was increased from medium to high. Interestingly, these values were not affected by the P supply (Table 3.2). The shoot Zn contents were also enhanced by not only higher Zn but also higher P applications. Significant increases in shoot P concentrations were observed in response to higher P supply. However, these increases were particularly pronounced in Zn-deficient plants. While there were no differences between the shoot P concentrations of medium Zn and high Zn plants, low Zn plants had markedly higher P concentrations, especially under high P conditions. The trends observed in shoot P contents were similar to those observed in shoot P concentrations, but the extents of the differences among the P treatments were even larger.

Table 3.2: Effect of low (20 μM P), adequate (100 μM P) and high (500 μM P) P, with three different Zn treatments as low (0.01 μM Zn), medium (0.1 μM Zn) and high (1 μM Zn), on shoot P and Zn concentrations and contents of 34-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants.

Zn Treat.	P Treat.	Zn Conc. (mg kg^{-1})	Zn Cont. (μg $plant^{-1}$)	P Conc. (% dry wt)	P Cont. (mg $plant^{-1}$)
Low	Low	5 ± 0	0.8 ± 0.0	0.25 ± 0.00	0.37 ± 0.00
	Adequate	5 ± 0	1.1 ± 0.1	0.74 ± 0.07	1.52 ± 0.20
	High	6 ± 1	1.1 ± 0.3	2.13 ± 0.17	3.55 ± 0.03
Medium	Low	22 ± 2	4.6 ± 0.4	0.19 ± 0.01	0.40 ± 0.02
	Adequate	19 ± 1	5.3 ± 0.2	0.52 ± 0.04	1.45 ± 0.06
	High	19 ± 1	5.4 ± 0.2	0.83 ± 0.05	2.36 ± 0.15
High	Low	48 ± 1	10.4 ± 0.5	0.18 ± 0.01	0.39 ± 0.01
	Adequate	46 ± 5	12.5 ± 2.2	0.51 ± 0.02	1.38 ± 0.13
	High	49 ± 6	14.5 ± 2.0	0.83 ± 0.04	2.47 ± 0.20

Zn Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (3; *n.s*; *n.s*)

Zn Cont. $HSD_{0.05}$ (Zn; P; ZnxP): (0.8; 0.8; 1.9)

P Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (0.07; 0.07; 0.16)

P Cont. $HSD_{0.05}$ (Zn; P; ZnxP): (0.12; 0.12; 0.29)

Both the Zn concentrations and contents were also markedly increased in roots in response to higher Zn applications (Table 3.3). The root Zn concentration was unaffected by P supply, whereas the root Zn content of Zn-sufficient plants was dramatically reduced when the P supply was increased from low to medium or high. Neither the root P concentration nor the root P content responded to Zn application, but both were significantly enhanced by higher P supply as expected.

Table 3.3: Effect of low (20 μM P), adequate (100 μM P) and high (500 μM P) P, with three different Zn treatments as low (0.01 μM Zn), medium (0.1 μM Zn) and high (1 μM Zn), on root P and Zn concentrations and contents of 34-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants.

Zn Treat.	P Treat.	Zn Conc. (mg kg^{-1})	Zn Cont. (μg $plant^{-1}$)	P Conc. (% dry wt)	P Cont. (mg $plant^{-1}$)
Low	Low	6 ± 0	0.6 ± 0.0	0.16 ± 0.00	0.18 ± 0.01
	Adequate	7 ± 1	0.6 ± 0.1	0.37 ± 0.09	0.30 ± 0.06
	High	8 ± 0	0.7 ± 0.1	0.71 ± 0.06	0.60 ± 0.09
Medium	Low	22 ± 2	2.0 ± 0.2	0.18 ± 0.01	0.17 ± 0.01
	Adequate	18 ± 1	1.3 ± 0.1	0.40 ± 0.01	0.29 ± 0.02
	High	20 ± 3	1.3 ± 0.2	0.80 ± 0.16	0.51 ± 0.11
High	Low	49 ± 3	5.1 ± 0.2	0.16 ± 0.01	0.17 ± 0.02
	Adequate	49 ± 3	3.1 ± 0.9	0.44 ± 0.10	0.27 ± 0.04
	High	51 ± 5	3.4 ± 0.5	0.80 ± 0.15	0.53 ± 0.10

Zn Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (3; *n.s*; *n.s*)

Zn Cont. $HSD_{0.05}$ (Zn; P; ZnxP): (0.2; 0.2; 0.5)

P Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (*n.s*; 0.09; *n.s*)

P Cont. $HSD_{0.05}$ (Zn; P; ZnxP): (*n.s*; 0.06; *n.s*)

The total Zn contents of wheat plants were tremendously enhanced by higher levels of Zn application. However, the P supply or its interaction with Zn supply did not have significant effects on the plant Zn content. When the Zn translocation index was calculated, it turned out that Zn-deficient plants transported a smaller portion of the Zn to their shoots than Zn-sufficient plants. Moreover, P deficiency under Zn-sufficient conditions was associated with a significantly reduced Zn translocation index.

Table 3.4: Effect of low (20 μM P), adequate (100 μM P) and high (500 μM P) P, with three different Zn treatments as low (0.01 μM Zn), medium (0.1 μM Zn) and high (1 μM Zn), on shoot, root and overall plant Zn contents of 34-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants.

Zn Treat.	P Treat.	Plant Zn Content (μg plant^{-1})	Zn Translocation Index (%)
Low	Low	1.4 ± 0.0	56 ± 2
	Adequate	1.7 ± 0.1	62 ± 3
	High	1.8 ± 0.2	58 ± 6
Medium	Low	6.6 ± 0.3	69 ± 4
	Adequate	6.7 ± 0.2	80 ± 1
	High	6.7 ± 0.1	81 ± 3
High	Low	15.5 ± 0.6	67 ± 1
	Adequate	15.6 ± 3.1	81 ± 3
	High	17.9 ± 2.4	81 ± 1

Plant Zn Cont. $HSD_{0.05}$ (Zn; P; ZnxP): (1.3; *n.s*; *n.s*)

Translocation Index $HSD_{0.05}$ (Zn; P; ZnxP): (3; 3; 7)

In the second experiment, major aim was to follow the impact of increasing P supply on root Zn uptake. In order to avoid severe stress situations with Zn or P deficiency (e.g., inhibition of root growth etc) and thus to avoid secondary effects on root Zn uptake the plants of this experiment were much younger than the first experiment. Accordingly, as shown in Table 3.5, the plant growth was not affected by the Zn supply. The shoot growth was also unaffected by the P application, whereas the root growth was reduced when the P supply was increased. Consequently, the root-to-shoot ratios of low P plants were significantly higher than those of adequate or high P plants.

Table 3.5: Shoot and root dry matter production of 20-day-old bread wheat (*Triticum aestivum* cv. Adana99), grown at low (20 µM P), adequate (100 µM P) and high (500 µM P) P, with two different Zn treatments as low (0.01 µM Zn) and high (1 µM Zn).

Zn Treat.	P Treat.	Shoot (mg plant^{-1})	Root (mg plant^{-1})	Root/Shoot (%)
Low	Low	83 ± 3	58 ± 4	71 ± 6
	Adequate	92 ± 8	55 ± 6	60 ± 2
	High	88 ± 6	50 ± 6	56 ± 7
High	Low	81 ± 5	58 ± 5	71 ± 3
	Adequate	90 ± 9	52 ± 4	58 ± 2
	High	84 ± 3	49 ± 2	58 ± 3

Shoot DW $HSD_{0.05}$ (Zn; P; ZnxP): (*n.s*; *n.s*; *n.s*)

Root DW $HSD_{0.05}$ (Zn; P; ZnxP): (*n.s*; 6; *n.s*)

Root/Shoot Ratio $HSD_{0.05}$ (Zn; P; ZnxP): (*n.s*; 5; *n.s*)

In this study, the shoot Zn concentrations and contents were quadrupled by the high Zn treatment, but they were at the same level in all P treatment groups. Similarly, the shoot P concentrations and contents were markedly enhanced by higher P treatments, but they were not affected by the Zn treatments.

Table 3.6: Effect of low (20 μM P), adequate (100 μM P) and high (500 μM P) P, with two different Zn treatments as low (0.01 μM Zn) and high (1 μM Zn), on shoot P and Zn concentration and content of 20-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants.

Zn Treat.	P Treat.	Zn Conc. (mg kg^{-1})	Zn Cont. (μg $plant^{-1}$)	P Conc. (%)	P Cont. (mg $plant^{-1}$)
Low	Low	14 ± 0	1.2 ± 0.1	0.30 ± 0.01	0.24 ± 0.01
	Adequate	13 ± 1	1.1 ± 0.0	0.79 ± 0.07	0.72 ± 0.06
	High	16 ± 2	1.4 ± 0.1	0.88 ± 0.11	0.78 ± 0.13
High	Low	67 ± 7	5.4 ± 0.8	0.29 ± 0.01	0.24 ± 0.02
	Adequate	58 ± 9	5.6 ± 0.6	0.74 ± 0.03	0.67 ± 0.05
	High	60 ± 4	5.1 ± 0.2	0.89 ± 0.04	0.75 ± 0.06

Zn Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (4; *n.s*; *n.s*)

Zn Cont. $HSD_{0.05}$ (Zn; P; ZnxP): (0.4; *n.s*; *n.s*)

P Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (*n.s*; 0.07; *n.s*)

P Cont. $HSD_{0.05}$ (Zn; P; ZnxP): (*n.s*; 0.08; *n.s*)

The Zn concentrations and contents measured in the roots of high Zn plants were about 6 times higher than those measured in the roots of low Zn plants. The Zn accumulation in the roots was not affected by the P supply. Both the root P concentration and the root P content results showed a marked increase in the P accumulation of roots at higher P supply levels. Zinc treatment did not have any effect on the P levels in roots.

Table 3.7: Effect of low (20 µM P), adequate (100 µM P) and high (500 µM P) P, with two different Zn treatments as low (0.01 µM Zn) and high (1 µM Zn), on root P and Zn concentration and content of 20-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants.

Zn Treat.	P Treat.	Zn Conc. (mg kg^{-1})	Zn Cont. (µg $plant^{-1}$)	P Conc. (%)	P Cont. (mg $plant^{-1}$)
Low	Low	13 ± 1	0.8 ± 0.0	0.25 ± 0.02	0.14 ± 0.01
	Adequate	13 ± 2	0.6 ± 0.0	0.51 ± 0.06	0.28 ± 0.04
	High	14 ± 2	0.7 ± 0.0	0.71 ± 0.09	0.35 ± 0.02
High	Low	78 ± 12	4.1 ± 0.3	0.27 ± 0.00	0.15 ± 0.01
	Adequate	88 ± 10	4.6 ± 0.8	0.60 ± 0.02	0.31 ± 0.03
	High	96 ± 7	4.7 ± 0.4	0.75 ± 0.02	0.37 ± 0.02

Zn Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (6; *n.s*; *n.s*)

Zn Cont. $HSD_{0.05}$ (Zn; P; ZnxP): (0.3; *n.s*; *n.s*)

P Conc. $HSD_{0.05}$ (Zn; P; ZnxP): (0.04; 0.05; *n.s*)

P Cont. $HSD_{0.05}$ (Zn; P; ZnxP): (0.02; 0.03; *n.s*)

The Zn uptake study revealed that low Zn plants absorbed Zn at higher rates than high Zn plants (Fig. 3.2). It was noted that Zn-deficient plants supplied with low P exhibited lower Zn uptake rates than those supplied with higher levels of P. Under Zn-sufficient conditions, however, the effect of P on the Zn uptake rate disappeared.

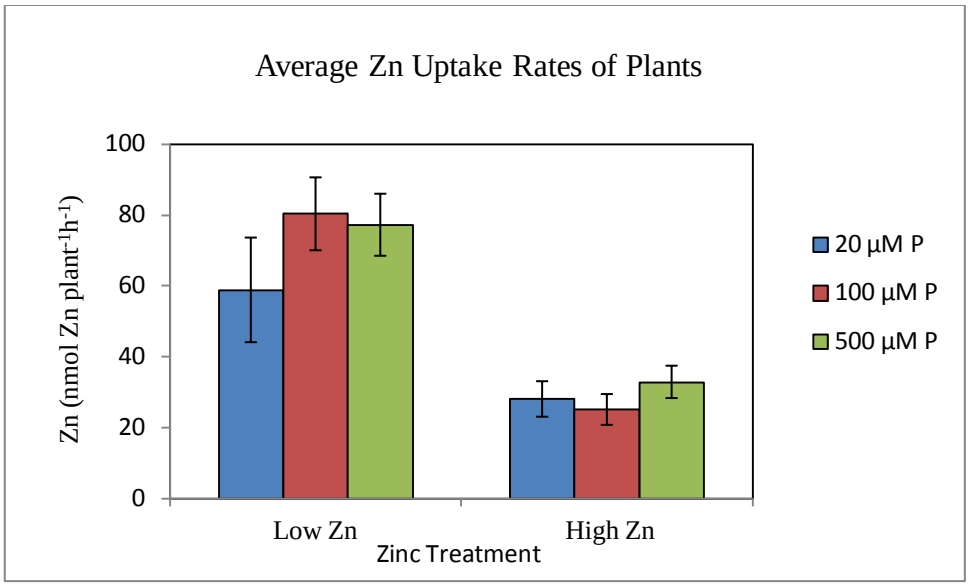


Fig.3.2: Effect of low (20 µM P), adequate (100 µM P) and high (500 µM P) P, with two different Zn treatments as low (0.01 µM Zn) and high (1 µM Zn), on the average Zn uptake rates of 20-day-old bread wheat (*Triticum aestivum* cv. Adana99).

The depletion of Zn from the nutrient solution was also followed. At the end of the experimental time interval (7 h), Zn-deficient plants supplied with adequate or high P had already absorbed almost all of the Zn in the solution, whereas Zn- and P-deficient plants depleted the Zn in the solution at a lower rate (Fig. 3.3A). Zinc was depleted at significantly lower rates from the solutions for high Zn plants (Fig. 3.3B). These results were also reflected in the cumulative Zn uptake data. Under high Zn conditions, the cumulative Zn uptake by wheat plants over the experimental time interval was low and unaffected by the P supply, whereas under low Zn conditions, the cumulative Zn uptake was markedly higher and also enhanced by adequate or high P supply (Fig. 3.3C, D). The cumulative Zn uptake was also calculated by taking the root biomass into account in order to exclude growth-related effects (Fig. 3.3E, F). The same trends were observed. So, the cumulative Zn uptake per unit root dry weight was reduced by low P supply in Zn-deficient plants. However, there was no significant difference between the adequate and high P treatments.

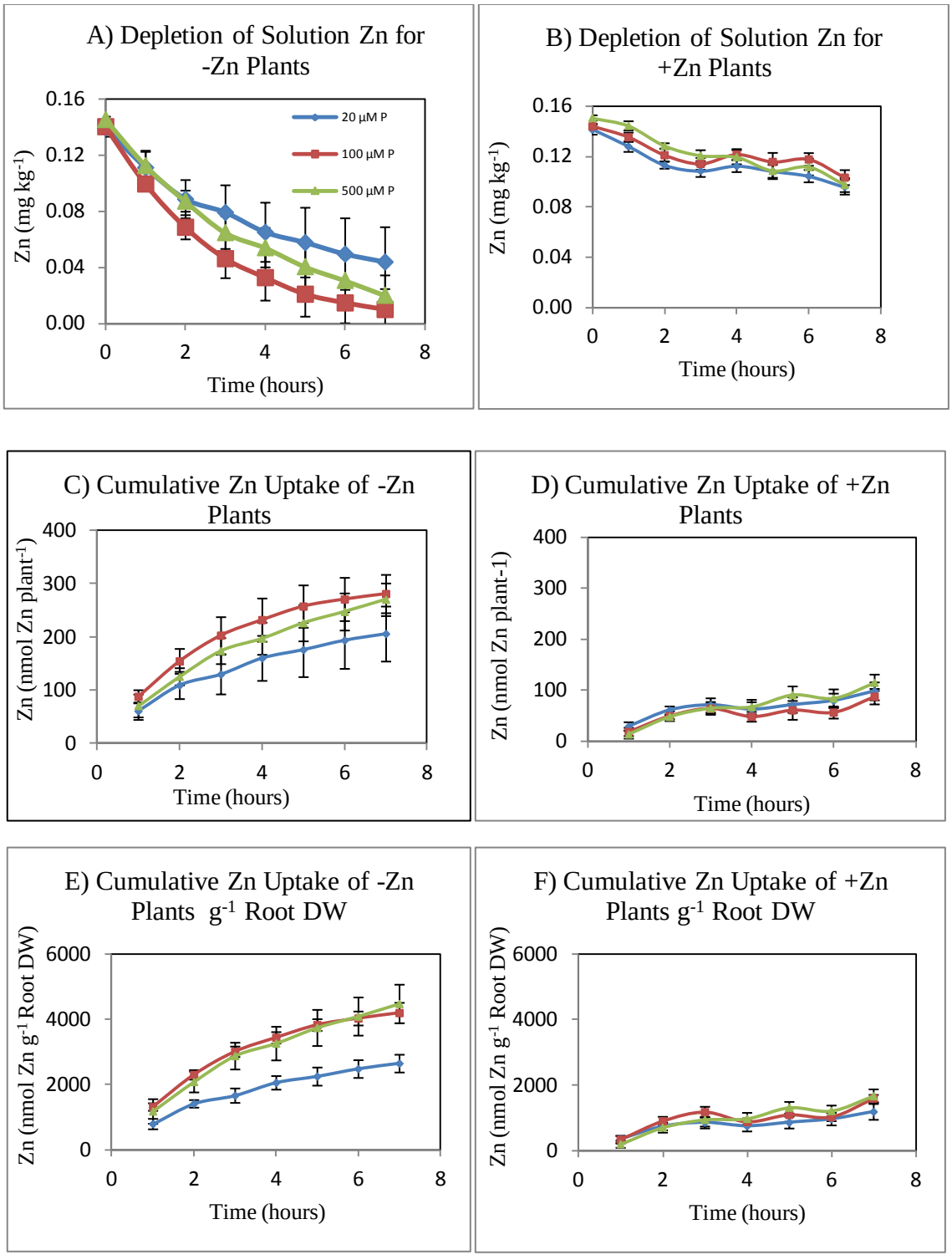


Fig.3.3: Effect of low (20 µM P), adequate (100 µM P) and high (500 µM P) P, with two different Zn treatments as low (0.01 µM Zn) and high (1 µM Zn), on the depletion of solution Zn for –Zn plants (A) and +Zn plants (B), cumulative Zn uptake of –Zn plants (C), and +Zn plants (D), cumulative Zn uptake per g root dry weight of –Zn plants (E), and +Zn plants (F), of 20-day-old bread wheat (*Triticum aestivum* cv. Adana99).

3.4. Discussion

In the first experiment, low Zn plants suffered from Zn deficiency and exhibited necrotic spots on their leaves. The severity of these symptoms was enhanced by higher P supply, but the Zn levels did not change with P treatment, indicating a reduction in Zn utilization efficiency. Similar observations were reported before (Cakmak and Marschner, 1987; Nichols et al., 2012). In the study of Cakmak and Marschner, this phenomenon was explained by a reduction of Zn solubility. Apart from that, the root dry matter was reduced with P application. The relatively high root-to-shoot ratio of P-deficient plants was reported before (Anuradha and Narayana, 1991) and considered as an adaptation mechanism of plants to P deficiency.

The P concentration and content of the shoot were increased by not only higher P supply but also by low Zn supply. This observation is in agreement with several reports in the literature where it was discussed as part of the antagonism between P and Zn. (Cakmak and Marschner, 1987; Huang et al., 2000; Barben et al., 2010; Nichols et al., 2012). However, since mycorrhizae were not present in the nutrient solution, this was clearly a physiological process. It was shown that in case of Zn deficiency, the expressions of high affinity phosphate transporter genes were induced in roots of barley plants, even if P reserves were sufficient (Huang et al., 2000).

Although the total Zn content of plants did not change with P treatment, the root-to-shoot translocation of Zn was significantly enhanced by higher P supply. So, the suggestion by Khanif and Saleem (2013) that high P can impair the shoot translocation of Zn was not supported by the present data.

In the second experiment, necrotic spots due to Zn deficiency were not observed because plants were grown under growth chamber conditions, where stress factors such as high temperature and excess light were minimized, and harvested earlier. As a result, the shoot dry weight was affected from neither P nor Zn treatments. Nevertheless, the root growth was affected by the P supply. The root-to-shoot ratio was reduced by higher P applications in conformity with the literature (Anuradha and Narayana, 1991).

In the Zn uptake experiment, plants grown with low Zn absorbed this element faster than plants grown with high Zn (Fig 3.1). This can be explained by a higher requirement of Zn-deficient plants for Zn. In addition, it was clear that high P status contributed significantly

to Zn uptake under Zn-deficient conditions. This observation seems to contradict the antagonism between Zn and P. The effect of P supply on the Zn uptake was not that clear at high Zn supply, since the uptake rates were dramatically reduced and the gaps between plants fed with different P levels were diminished. Nevertheless, also at high Zn, low P plants exhibited lower Zn absorption rates than higher P plants. Based on these findings, the following can be suggested about the interaction between Zn and P under hydroponics conditions: When plants are young and small, their Zn requirement is also low. However, plants supplied with low Zn can nevertheless absorb all the Zn available in the nutrient solution before the solution is refreshed, irrespective of the P supply. In contrast, at high Zn supply, the time between solution refreshments may not be enough for plants to absorb all the available Zn, and then high P plants may have an advantage in accumulating Zn. In this study, it was reflected as an increase in root Zn levels at high Zn. However, in the first experiment (Table 3.5), there was no significant difference observed in root Zn levels of plants fed with different P levels. As plants grew, their Zn demand also increased. Probably, all plants were able to take up all the Zn from the nutrient solution, so that the differences due to P nutrition lost their importance under these conditions.

3.5. Conclusions

Under solution culture conditions, P and Zn do not exhibit an antagonistic interaction, which supports the idea that such interaction observed commonly in soil-grown plants is actually due to the effect of mycorrhiza. The results presented in this chapter indicate that a sufficiently high P nutrition can enhance the root uptake as well as the root-to-shoot translocation of Zn. A high P supply can then have a positive impact on the Zn nutrition of plants. However, under soil conditions, the suppression of mycorrhiza by higher P supply exerts a dominant negative effect on the Zn uptake.

(C) GENERAL DISCUSSION AND CONCLUSIONS

Micronutrient deficiencies in human populations are growing global health issue affecting approximately one third of world population, especially in developing countries (Welch & Graham, 2004; Cakmak et al., 2010). Among all micronutrient deficiencies, Zn deficiency has a particular importance because it can lead to the emergence of severe health issues such as susceptibility to infectious diseases, stunting, delayed bone maturation, impaired sexual and cognitive development, increased morbidity and mortality (Welch and Graham, 2004; Gibson et al., 2008).

Biofortification of staple crops with Zn by either agronomic approaches or plant breeding strategy represent a powerful and efficient tool to alleviate the problem. In order to use agronomic biofortification efficiently, there is a high need to understand the nature and interaction of mineral nutrients when applied in soil. Nitrogen and P are most commonly applied fertilizers. In case of N, several reports demonstrated that N and Zn are synergistic during their root uptake and seed deposition (Kutman et al., 2011; Cakmak et al., 2010), whereas high P applications have the potential to lower shoot and grain Zn concentration (Ryan et al., 2008; Lu et al., 2011; Zhang et al., 2012). It is therefore important to collect further information about those nutrient interactions. In case of Zn and N interaction, number of papers have been published and convincing evidence about synergism between Zn and N has been documented (see Cakmak et al., 2010; Kutman et al., 2011 and 2012).

In case of P and Zn interaction there is extensive amount of contradictory results. It seems that growth medium used in those studies, soil medium or nutrient solution might be a major reason for the reported controversial results on P and Zn interactions. Most relevant difference between these growth conditions are the absence of mycorrhiza in nutrient solution culture. In case of soil tests, impact of mycorrhizae on P-Zn interaction was not adequately studied because the tests conducted did not involve sterilization of the experimental soils in which P-Zn interaction was specifically studied.

In this thesis, 3 different experiments were conducted in which plants were grown in soils with increasing P and Zn applications with or without soil sterilization (Chapters I and II) or only in nutrient solution (Chapter-III). The results clearly demonstrated that lack of mycorrhiza represents an important contributing factor to P-induced Zn deficiency and very low Zn concentrations of plants (in shoot or grain). It is known that high P diminishes mycorrhizae activity and thus limits contribution of mycorrhizae to Zn nutrition of plants. This P or mycorrhizae effect on Zn is very specific because absence or presence of mycorrhizae (achieved through differential soil sterilization) did not change concentrations of many other mineral nutrients in plants. Consequently, these results did not support the idea that Zn may be precipitated in the soil when P is applied at high rates. In well agreement with this suggestion, nutrient solution tests showed that increases in P concentration in solution was not able to reduce root Zn uptake and even it promoted root Zn uptake.

In order to achieve positive contributions of mycorrhizae to Zn uptake of plants maintenance of high mycorrhizae activity in rhizosphere is of great importance. Mycorrhizal population and activity in rhizosphere are very much affected from level of soil organic matter and intensive usage of soil for agricultural purposes (e.g., soil degradation). Under such soil conditions, agronomic practices should be taken into consideration to improve and maintain high mycorrhizal fungus population and their activity in rhizosphere such as addition of organic matter and mycorrhizal inoculums (or commercial preparates) into soils.

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