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REGULAR ARTICLE

High phosphorus supply reduced zinc concentration of wheat in native soil but not in autoclaved soil or nutrient solution

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Abstract
Aims Phosphorus (P)-induced zinc (Zn) deficiency is one of the most commonly studied antagonistic interactions in plant nutrition. However, there are many controversial reports about P–Zn interaction, possibly related to growth conditions. In this study, the effects of P supply on the root uptake and tissue concentrations of Zn as well as the development of Zn deficiency were investigated in wheat (*Triticum aestivum*) grown in different media.
Methods Plants were grown under greenhouse and growth chamber conditions in native soil, autoclaved soil and nutrient solution with different P and Zn supplies. In the soil experiment, the shoot biomass and grain yield were measured whereas in the nutrient solution experiment, the root and shoot biomass were determined. Development of Zn deficiency symptoms was examined. Concentrations of Zn, P and other elements were measured in harvested tissues. Mycorrhizal colonization of roots was scored in soil-grown plants. Root uptake of stable Zn isotope (^{70}Zn) was investigated at different P rates in a separate nutrient solution experiment.
Results Higher P rates caused substantial decreases in shoot and grain Zn concentrations in native soil but not in autoclaved soil. Treatment of native soil with increasing P significantly reduced mycorrhizal colonization. At low Zn, P applications aggravated Zn deficiency symptoms in both soil and solution culture. In solution culture, root and shoot Zn concentrations were not lowered by higher P rates. Root uptake of ^{70}Zn from nutrient solution was even depressed at low P.
Conclusions The negative effect of increasing P supply on root Zn uptake and tissue Zn concentrations in wheat is mycorrhiza-dependent and may completely disappear in a mycorrhiza-free environment.

Keywords Arbuscular mycorrhiza (AM) · Autoclaved soil · Nutrient solution · Phosphorus · Wheat (*Triticum aestivum*) · Zinc deficiency

Introduction

As early as 1970s, phosphorus (P) and zinc (Zn) were documented to interact with each other in plant mineral nutrition (Marschner and Schropp 1977; Loneragan et al. 1979; Warnock 1970). Although this interaction is commonly referred to as "P-induced Zn deficiency", it is more complex than this term implies (Marschner 2012). In soil-grown plants, higher levels of P often reduce the Zn concentrations in both vegetative tissues and seeds. If the Zn availability is low, this effect can cause or exacerbate Zn deficiency. Under both controlled and field conditions, higher soil P applications were associated with lower tissue Zn concentrations in wheat (*Triticum aestivum*) (Thompson 1990; Zhu et al. 2001; Zhang et al. 2012) as well as numerous other crops (Singh et al. 1988; Li et al. 2003; Broadley et al.

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2010). Intriguingly, the negative effect of higher P supply on tissue Zn levels was not observed in solution culture studies (Cakmak and Marschner 1986; Nichols et al. 2012).

Zinc deficiency is also associated with elevated shoot P levels and can cause P toxicity at high P supplies (Marschner 2012). The basipetal transport of P, which normally acts as a feedback mechanism regulating P uptake, was shown to be specifically inhibited by Zn deficiency in cotton (*Gossypium hirsutum*) (Marschner and Cakmak 1986). Zinc deficiency also induces the expression of genes encoding P uptake transporters as demonstrated in barley (*Hordeum vulgare*) (Huang et al. 2000). Elevations in shoot P concentrations due to Zn deficiency can in turn aggravate Zn deficiency. In corn (*Zea mays*), high levels of P can immobilize Zn in roots and nodes by increasing the Zn-binding properties of the cell wall (Dwivedi et al. 1975; Youngdahl et al. 1977). Supporting these findings, reduced translocation of Zn from root to shoot tissues was reported to contribute to P-induced Zn deficiency in bean (*Phaseolus vulgaris*) (Singh et al. 1988). Elevated tissue P levels may reduce the water-soluble Zn concentration and thus lower the physiological availability of Zn (Rahimi and Schropp 1984; Cakmak and Marschner 1987). Moreover, P toxicity as an additional stress factor accentuates symptoms and worsens the condition of Zn-deficient plants (Loneragan et al. 1982; Cakmak and Marschner 1986; Webb and Loneragan 1988).

Increased root-to-shoot biomass ratio due to impaired shoot growth and relatively less affected or even stimulated root growth is a typical response of plants to P deficiency (Anuradha and Narayanan 1991; Cakmak et al. 1994; Watts-Williams et al. 2013). Under P-limited conditions, improvements in P nutritional status may "dilute" Zn in the above-ground parts of plants by promoting the shoot growth while reducing the root growth and thus the Zn acquisition capacity (Marschner 2012). However, in the literature, results related to P-induced Zn deficiency could rarely be explained by the dilution effect alone (Gianquinto et al. 2000). In most studies, although the dilution effect was taken into account as a possibly contributing factor, the negative effects of higher P supply on tissue Zn concentrations and Zn deficiency symptoms could not be simply ascribed to dilution (Lambert et al. 1979; Loneragan et al. 1979; Singh et al. 1988; Li et al. 2003; Zhang et al. 2012).

Chemical interactions between Zn and P species in the growth medium have also been discussed as a possible reason of P-induced Zn deficiency in plants (Verma and Minhas 1987; Marschner 2012). Loneragan et al. (1979) documented that high P applications depressed the Zn uptake of clover (*Trifolium subterraneum*) from ferruginous sand but not from siliceous sand, suggesting an interaction between P and Zn dependent on soil chemistry. It was also shown that phosphate could form complexes with Zn on colloid surfaces in acidic soils rich in Fe and Al oxides and thus boost Zn adsorption to soil particles (Perez-Novo et al. 2011). However, the practical relevance of such P-Zn interactions in soils is not clear. In a field study on wheat, where higher P applications significantly reduced grain Zn levels, the DTPA-extractable Zn concentration of the soil did not vary with P applications (Zhang et al. 2012).

Arbuscular mycorrhiza (AM), which enables the host plant to extract P from soil beyond the rhizosphere depletion zone, is also known to contribute to the Zn (and Cu) uptake of the plant significantly (Kothari et al. 1991; Li et al. 1991; Tinker et al. 1992; Smith and Read 2008; Ortas 2012). A meta-analysis conducted on 104 research articles including 263 trials showed that AM positively contributes to shoot and seed concentrations of Zn in different plants (Lehmann et al. 2014). The well-documented suppression of mycorrhizal colonization by high P could therefore adversely affect the Zn nutritional status of plants (Lambert et al. 1979; Loneragan and Webb 1993; Marschner 2012; Watts-Williams et al. 2013) and has been a common issue in research on P–Zn interaction. A pot study on soybean (*Glycine max*) and corn grown in steam-pasteurized soil revealed that higher levels of P reduced the shoot Zn and Cu concentrations when the soil was inoculated with AM fungi whereas this effect was less pronounced or absent in non-inoculated soil (Lambert et al. 1979). Similarly, in a pot experiment with wheat, the Zn uptake and tissue Zn concentration were reduced by P application in the presence of AM colonization but unaffected in its absence (Ryan and Angus 2003). The negative effect of P applications on the grain Zn concentration of wheat was also documented in several field studies (Ryan and Angus 2003; Ryan et al. 2008; Zhang et al. 2012). Phosphorus application impaired the root AM colonization, which correlated well with the grain Zn concentration (Ryan and Angus 2003; Ryan et al. 2008). Reductions in grain Zn by P applications may have adverse impacts on human Zn nutrition in countries where cereals are main source of daily calorie intake

(Cakmak 2008). Zinc deficiency is one of the well-documented micronutrient deficiencies occurring in human populations with serious health complications (Krebs 2013).

Despite extensive research on P–Zn interaction in wheat, it has still not been clarified which of these mechanisms is primarily responsible for the observed negative effects of high P applications on the tissue Zn levels. An improved understanding of this phenomenon may also have important implications for Zn biofortification efforts in cereals. In order to test the hypothesis that P-induced Zn deficiency is mycorrhiza-dependent, P effects on tissue Zn concentrations were investigated both in autoclaved vs. non-autoclaved soil. The same interaction was also examined in solution culture, which is free from AM activity. In addition, the effect of increasing P treatments on the root uptake and root-to-shoot translocation of Zn in the absence of AM was studied in solution culture by using the stable ^{70}Zn isotope. To our knowledge, this is the first study where the P–Zn interaction was examined in soil- and hydroponically-grown wheat at the same time.

Materials and methods

In this study, one soil and one solution culture experiment were conducted simultaneously under greenhouse conditions to investigate the Zn–P interaction in bread wheat (*Triticum aestivum* cv. Adana99). Then, a second solution culture experiment was performed in a growth chamber to further investigate the effect of P on root Zn uptake in solution culture under controlled conditions.

Greenhouse and growth chamber conditions

The greenhouse used in this study is a Venlo-type greenhouse located in Istanbul at the following geographic coordinates: 40° 53′ 25″ N, 29° 22′ 47″ E. It has computerized climate control (heating and evaporative cooling) and supplemental lighting. During this study, the daytime temperature was kept at 25±4 °C and the night time temperature at 20±4 °C in the greenhouse.

In the growth chamber, during the daytime (16 h), the photosynthetic flux density was 400 μmol m^{-2} s^{-1}, the temperature was 24 °C, and the relative humidity was 60 %. During the night, the temperature was 22 °C, and the relative humidity was 70 %.

Soil experiment

For studying the Zn–P interaction under both Zn-deficient and Zn-sufficient soil conditions, a Zn-deficient soil of Central Anatolian origin was used in this experiment. It was a calcareous (18 % $CaCO_3$) and alkaline (pH 8.0 in dH_2O) clay loam with low organic matter (1.5 %). The diethylenetriaminepentaacetic acid (DTPA)-extractable Zn concentration of the untreated soil was 0.13 mg kg^{-1} according to the method described by Lindsay and Norvell (1978).

One half of the experimental soil was autoclaved at 121 °C for 2 h in order to eliminate the native mycorrhizal fungi and then air-dried. Pots were filled with 3.1 kg of either non-autoclaved or autoclaved soil. Before the seeds were sown, the following mineral nutrients were added to each pot as concentrated solutions and uniformly incorporated into the soil my mixing thorougly (per kg air-dry soil): 300 mg N in the form of $Ca(NO_3)_2$ $4H_2O$; 25 mg S in the form of K_2SO_4; 15 mg (low), 60 mg (medium) or 180 mg (high) P in the form of $Ca(H_2PO_4)_2$; and 0.2 mg (low) or 5.0 mg (high) Zn in the form of $ZnSO_4$ $7H_2O$. The soil experiment had a fully factorial design with 2 sets of 5 pot replicates per treatment, and the pots were completely randomized in the greenhouse. Seven plants were grown in each pot. Throughout the experiment, the pots were watered daily with deionized water. The pots were supplied with additional 100 mg N in the form of $Ca(NO_3)_2$ $4H_2O$ 52 days after sowing (DAS).

At the beginning of the experiment, samples were taken from the fertilized soils (both non-autoclaved and autoclaved), and their DTPA-extractable Zn, iron (Fe), copper (Cu) and manganese (Mn) concentrations were determined. The extractable Zn concentrations of the treated soils ranged from 0.17 to 0.20 mg kg^{-1} in the low-Zn group and from 2.32 to 2.44 mg kg^{-1} in the high-Zn group. Neither autoclaving nor P applications had any effect on the extractable Zn concentration. None of the treatments affected the extractable concentrations of Fe and Cu, which ranged from 2.83 to 3.04 mg kg^{-1} and from 0.62 to 0.65 mg kg^{-1}, respectively. However, autoclaving greatly enhanced the extractable Mn concentration (from 1.22 to 6.52 mg kg^{-1} on average), and higher P applications reduced the extractable Mn by up to 20 %.

At booting (66 DAS), plants in the first set of pots were harvested, and samples from their roots were used to assess mycorrhizal colonization as described below.

For analyzing the shoot dry matter production and shoot mineral concentrations in developing plants, two plants were harvested at anthesis (81 DAS) from each pot in the second set by cutting them off at the soil level. The remaining five plants were harvested at maturity. Harvested shoots were dried for 2 days at 65 °C, weighed and analyzed for mineral nutrient concentrations as described in "Digestion and mineral analyses".

Solution culture experiments

Seeds were germinated in perlite moistened with a saturated $CaSO_4$ solution for 5 days at room temperature. Then, seedlings were transferred to 3-L pots containing a nutrient solution with the following composition: 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 $(NH_4)_6Mo_7O_{24}.4H_2O$. Different levels of P in the form of $Ca(H_2PO_4)_2$ and Zn in the form of $ZnSO_4$ $7H_2O$ were added to the nutrient solutions, depending on the treatment. Nutrient solutions were continuously aerated and refreshed three times a week. Both solution culture experiments had fully factorial designs with four pot replicates per treatment, and the pots were completely randomized.

In the first solution culture experiment, 20 plants were grown in each pot. The plants were supplied with 20 μM (low), 100 μM (medium) or 500 μM (high) P and 0.01 μM (low), 0.1 μM (medium) or 1 μM (high) Zn. When the plants were 34 days old, their shoots and roots were harvested.

The second solution culture experiment was the ^{70}Zn uptake experiment. Here, ten plants were grown in each pot. They were supplied with 20 μM (low), 100 μM (medium) or 500 μM (high) P and either 0.01 μM (low) or 1 μM (high) Zn. When they were 19 days old, the ^{70}Zn uptake study was performed on two randomly selected plants from each pot. They were transferred to Erlenmeyer flasks filled with 300 ml of dilute (1:10) nutrient solution and kept there for 30 min. Then, they were transferred to another set of flasks containing 2 μM ^{70}Zn plus all macronutrients at standard levels but no other micronutrients in 300 ml. In order to study the rate of ^{70}Zn depletion from the solution, 3 ml samples were taken from the flasks at 0 h (just before transferring the plants to the flasks), 1 h, 2 h, 3 h, 5 h and finally 7 h. After the last sampling, the plants were once again transferred to new flasks filled with 300 ml of dilute (1:10) nutrient solution. They were kept there for 30 min and then put back to their original pots, where they were grown for another day. So, the plants had 24 h to translocate the ^{70}Zn they took up from the root to the shoot. The experiment was then terminated by harvesting the shoots and roots separately.

In both solution culture experiments, the harvested roots were washed thoroughly by sequentially dipping them in dH_2O, 1 mM $CaCl_2$, 1 mM EDTA and finally again dH_2O. All shoot and root samples were dried for 2 days at 65 °C, weighed and analyzed for mineral nutrient concentrations as described in "Digestion and mineral analyses".

Measurement of mycorrhizal colonization

In harvested roots, mycorrhizal colonization was evaluated according to the "slide" method described by Giovanetti and Mosse (1980). The roots were washed with dH_2O in order to remove the soil particles. About 3 cm-long root tips were cut and preserved in ethanol until further processing. First, the root tips were immersed in 10 % (*w/v*) KOH and kept at 65 °C for 1 h. Then, the KOH solution was discarded, and the samples were treated with 10 % HCl (*v/v*) at 65 °C for 15 min. After discarding the HCl solution, the root tips were treated with 0.05 % (*w/v*) trypan blue solution at 65 °C for 25 min. The processed root samples were preserved in lactic acid until microscopic evaluation of mycorrhizal colonization. For each sample, ten pieces of root tips were arranged under the microscope. Root tips were used to focus on a standard part of the root system. Presence or absence of colonization was recorded for each of the 10 pieces and thus each sample was given a score between 0 (no sign of infection) and 10 (heavily infected).

Digestion and mineral analyses

All dried shoot or grain samples were ground to fine powders in an agate vibrating cup mill (Pulverisette 9; Fritsch GmbH; Germany). Ground samples (~0.2 g) were digested in a closed-vessel microwave system (MarsExpress; CEM Corp; Matthews, NC, USA) with 2 ml of 30 % H_2O_2 and 5 ml of 65 % HNO_3.

In soil DTPA extracts, digested plant samples and nutrient solution samples containing ^{70}Zn as the only Zn isotope, the concentrations of mineral nutrients

including Cu, Fe, Mg, Mn, P and Zn were measured by inductively coupled plasma optical emission spectrometry (ICP-OES; Vista-Pro Axial; Varian Pty Ltd; Mulgrave, Australia). For measuring the ^{70}Zn concentration in digested plant samples, inductively coupled plasma mass spectrometry (ICP-MS; Agilent; CA, USA) was used. Measurements were checked by using certified standard reference materials obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA).

Statistical analyses

The JMP software (Version 11.0.0) was used for statistical analyses. Analysis of variance (ANOVA) was conducted to evaluate the significance of the effects of the treatments and their interactions. When effects were significant according to ANOVA, significant differences between means were determined by Tukey's honestly significant difference (HSD) test ($P<0.05$).

Results

The shoot dry weights of 81-day-old wheat plants grown in soil culture were significantly affected by soil sterilization as well as Zn and P applications, but no significant interactions were observed (Table 1). On average, soil sterilization and high soil Zn increased the shoot biomass by 14 % and 7 %, respectively. With respect to the low-P treatment, the medium-P treatment enhanced the shoot biomass production by 50 %, but the high-P application did not provide any additional benefit. As can be seen in Fig. 1, the general condition and visual appearance of the plants varied greatly according to the treatments at this stage. Under non-autoclaved soil conditions (Fig. 1a), when the Zn supply was low, increasing P supply resulted in reduced plant height, delayed development and caused more severe leaf symptoms including chlorosis and necrosis. In contrast, when the Zn supply was high, higher P applications increased plant height and overall vigor. Similar trends were also observed in autoclaved soil (Fig. 1b), but the effects were less pronounced. In terms of grain yield, the interactive effects of soil Zn and P applications were significant at maturity (Table 1). They markedly augmented the positive effects of each other on grain yield. At low P, the grain yield did not respond to high Zn, whereas at high P, plants produced 50 % more grains by weight in response to high Zn.

In roots of 66-day-old wheat plants grown in non-autoclaved soil, the extent of mycorrhizal colonization

Table 1 Effects of Zn (low: 0.2 mg kg^{-1}; high: 5 mg kg^{-1}) and P (low: 15 mg kg^{-1}; medium: 60 mg kg^{-1}; high: 180 mg kg^{-1}) applications and soil sterilization (autoclaving) on the shoot dry weight of 81-day-old and grain yield of mature bread wheat (*Triticum aestivum* cv. Adana99) grown under greenhouse conditions

Soil Zn (A)	Sterilization (B)	Soil P (C)	Shoot DW (g $plant^{-1}$)	Grain yield (g $plant^{-1}$)
Low	None	Low	3.3±0.5	2.6±0.2
		Medium	5.1±0.4	4.2±0.3
		High	5.1±0.4	3.9±0.2
	Autoclaved	Low	3.8±0.5	2.6±0.4
		Medium	5.6±0.7	4.5±0.3
		High	5.5±0.6	4.6±0.7
High	None	Low	3.4±0.3	3.0±0.3
		Medium	5.0±0.4	5.2±0.4
		High	5.7±0.7	6.4±0.8
	Autoclaved	Low	4.2±0.6	2.5±0.3
		Medium	6.2±0.5	5.8±0.4
		High	5.9±0.9	6.5±0.9

Values are means and standard deviations of five pot replicates

$HSD_{0.05}$ values: A (soil Zn); B (soil sterilization); C (soil P); A × B; A × C; B × C; A × B × C

Shoot DW: 0.3; 0.3; 0.4; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Grain yield: 0.3; *n.s.*; 0.4; *n.s.*; 0.7; *n.s.*; *n.s.*

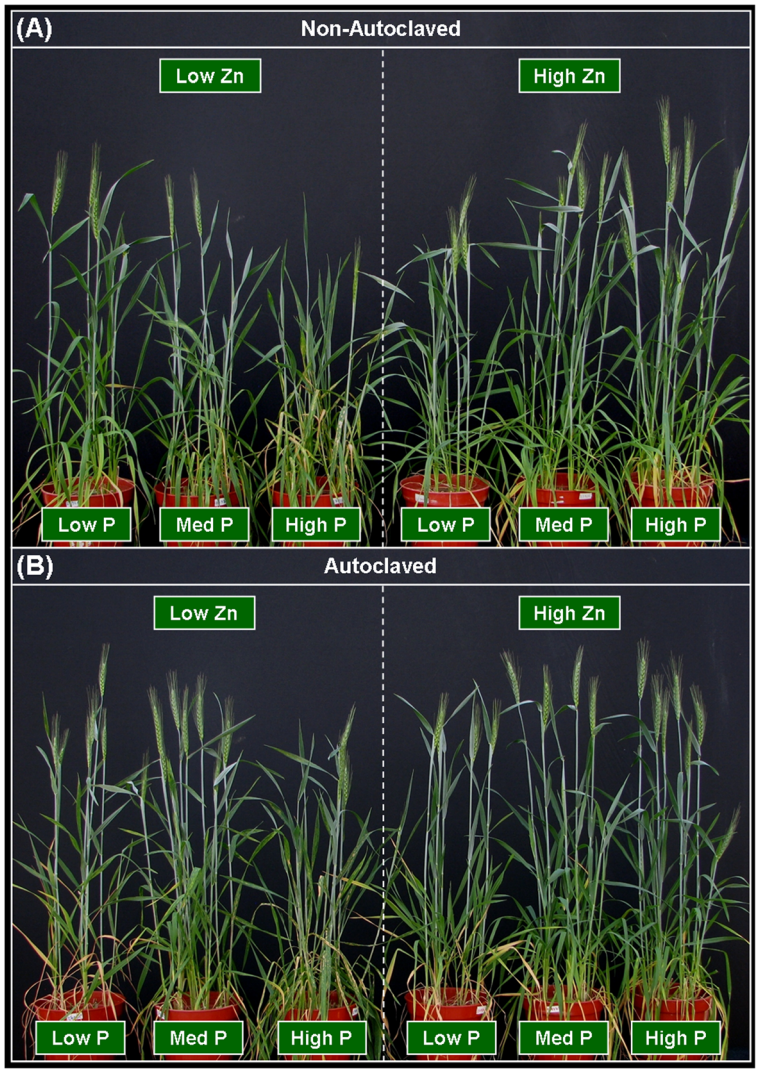

Fig. 1 81-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants grown in **a** non-autoclaved or **b** autoclaved soil with varied Zn (low: 0.2 mg kg^{-1}; high: 5 mg kg^{-1}) and P (low: 15 mg kg^{-1}; medium: 60 mg kg^{-1}; high: 180 mg kg^{-1}) applications under greenhouse conditions

depended only on the P supply (Fig. 2). With each increase in P rate, a dramatic decrease was observed in the mycorrhizal colonization score under both the low-Zn and high-Zn conditions. In plants grown in autoclaved soil, mycorrhizal colonization was not detectable at booting (data not shown). The shoot Zn concentration at anthesis was significantly affected by the triple interaction of the soil sterilization, Zn and P treatments (Table 2). In non-autoclaved soil, the low-P plants had markedly higher shoot Zn concentrations than the medium-P and high-P plants at both Zn supply levels. This effect of P supply on the shoot Zn concentration disappeared completely in autoclaved soil where the shoot Zn concentrations of the high-Zn plants were 4–5 times as high as those of the low-Zn plants, irrespective of the P level. In terms of the shoot P concentration, the interaction of soil sterilization and P application appeared to have the most important effect. Higher soil P applications resulted in remarkably higher shoot P concentrations in any case. Autoclaving the soil reduced the shoot P concentration by 35 % at the low P level, but did not have any significant effect on it at the medium and high P levels.

In parallel with the shoot P concentration, the shoot Mg concentration decreased significantly with decreasing soil P supply in developing wheat plants (Table 2). Similarly, the lowest shoot Cu concentrations were measured in the low-P plants. The shoot Mn levels were dramatically altered by the interactive effects of the treatments. Both autoclaving soil and higher P

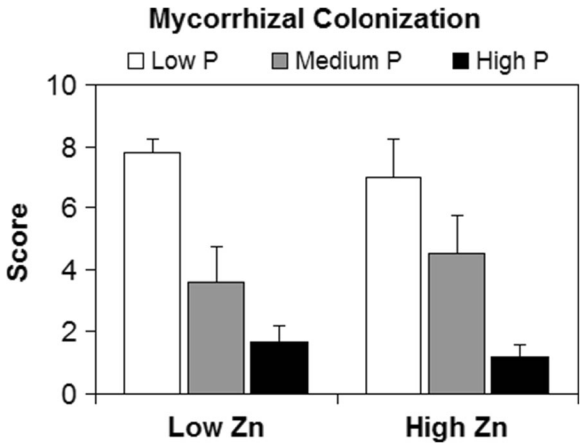

Fig. 2 Effects of Zn (low: 0.2 mg kg^{-1}; high: 5 mg kg^{-1}) and P (low: 15 mg kg^{-1}; medium: 60 mg kg^{-1}; high: 180 mg kg^{-1}) applications on the mycorrhiza infection scores of 66-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants grown in non-autoclaved soil under greenhouse conditions. Plotted values are means and standard deviations of 5 pot replicates. *Different letters* above columns indicate significantly different means according to Tukey's HSD test ($p<0.05$)

application resulted in elevated shoot Mn concentrations. However, the effects of P on shoot Mn were particularly prominent in non-autoclaved soil. At high Zn, the shoot Mn levels were lower, and the effects of soil autoclaving and P supply were weaker than at low Zn.

The grain Zn concentrations varied significantly in response to the treatments (Table 3). In non-autoclaved soil, the grain Zn concentration decreased markedly with increasing soil P supply at both Zn supply levels, whereas in autoclaved soil, the P supply did not have any clear effect on the grain Zn. The grain P concentration did not show a clear response to the Zn supply. While higher P supply enhanced the grain P significantly, the effects of soil autoclaving depended on the P supply. At high P, soil autoclaving did not affect the grain P concentration, but at medium and low P, autoclaving caused significant decreases in the grain P, and these were particularly pronounced at low P. In terms of grain concentrations, Mg and Cu exhibited similar trends. The grain concentrations of both were

Table 2 Effects of Zn (low: 0.2 mg kg^{-1}; high: 5 mg kg^{-1}) and P (low: 15 mg kg^{-1}; medium: 60 mg kg^{-1}; high: 180 mg kg^{-1}) applications and soil sterilization (autoclaving) on the shoot Zn, P, Mg, Cu and Mn concentrations of 81-day-old bread wheat (*Triticum aestivum* cv. Adana99) grown under greenhouse conditions

Soil Zn (A)	Sterilization (B)	Soil P (C)	Shoot mineral concentrations				
			Zn (mg kg^{-1})	P (%)	Mg (%)	Cu (mg kg^{-1})	Mn (mg kg^{-1})
Low	None	Low	11.3±0.8	0.15±0.01	0.14±0.01	4.5±0.5	71±4
		Medium	6.7±0.9	0.24±0.04	0.23±0.02	6.3±0.6	117±7
		High	5.5±0.4	0.32±0.00	0.26±0.01	7.7±1.2	154±2
	Autoclaved	Low	7.2±0.8	0.10±0.01	0.14±0.01	4.7±0.4	147±10
		Medium	6.0±0.5	0.20±0.01	0.21±0.01	6.7±0.2	178±8
		High	5.0±0.4	0.29±0.02	0.23±0.02	7.0±0.2	189±21
High	None	Low	40.6±1.6	0.15±0.01	0.15±0.01	5.4±0.4	74±8
		Medium	28.1±1.1	0.20±0.02	0.22±0.02	5.9±0.4	96±6
		High	26.1±1.4	0.31±0.02	0.26±0.03	6.0±0.3	98±8
	Autoclaved	Low	27.7±1.8	0.10±0.00	0.16±0.01	4.2±0.1	133±5
		Medium	29.0±1.5	0.20±0.01	0.22±0.02	6.1±0.5	132±14
		High	25.9±2.0	0.31±0.02	0.23±0.02	5.9±0.5	132±13

Values are means and standard deviations of five pot replicates

$HSD_{0.05}$ values: A (soil Zn); B (soil sterilization); C (soil P); A × B; A × C; B × C; A × B × C

Shoot Zn Concentration: 6.3; 6.3; 0.9; 1.2; 1.6; 1.6; 2.7

Shoot P Concentration: *n.s.*; 0.01; 0.01; 0.02; *n.s.*; 0.02; *n.s.*

Shoot Mg Concentration: *n.s.*; 0.01; 0.01; *n.s.*; *n.s.*; 0.02; *n.s.*

Shoot Cu Concentration: 0.3; *n.s.*; 0.4; *n.s.*; 0.7; 0.7; 1.1

Shoot Mn Concentration: 5; 5; 8; 10; 13; 13; *n.s.*

Table 3 Effects of Zn (low: 0.2 mg kg^{-1}; high: 5 mg kg^{-1}) and P (low: 15 mg kg^{-1}; medium: 60 mg kg^{-1}; high: 180 mg kg^{-1}) applications and soil sterilization (autoclaving) on the grain Zn, P, Mg, Cu and Mn concentrations of bread wheat (*Triticum aestivum* cv. Adana99) grown under greenhouse conditions

Soil Zn (A)	Sterilization (B)	Soil P (C)	Grain mineral concentrations				
			Zn (mg kg^{-1})	P (%)	Mg (%)	Cu (mg kg^{-1})	Mn (mg kg^{-1})
Low	None	Low	32±5	0.27±0.02	0.14±0.01	6.2±0.2	39±2
		Medium	12±1	0.38±0.02	0.16±0.00	6.8±0.5	54±3
		High	10±1	0.44±0.02	0.17±0.01	7.4±0.3	67±4
	Autoclaved	Low	12±1	0.17±0.01	0.10±0.00	4.9±0.3	43±5
		Medium	8±0	0.32±0.02	0.15±0.01	6.8±0.4	68±4
		High	8±1	0.44±0.01	0.16±0.00	7.1±0.3	78±4
High	None	Low	60±1	0.24±0.01	0.13±0.00	7.0±0.3	35±3
		Medium	48±5	0.35±0.03	0.16±0.01	6.8±0.5	49±3
		High	37±6	0.44±0.01	0.17±0.01	6.3±0.5	51±4
	Autoclaved	Low	36±3	0.17±0.01	0.11±0.00	5.5±0.5	42±2
		Medium	37±3	0.29±0.02	0.14±0.01	6.6±0.4	54±4
		High	36±5	0.43±0.01	0.16±0.01	6.4±0.8	61±3

Values are means and standard deviations of five pot replicates

$HSD_{0.05}$ values: A (soil Zn); B (soil sterilization); C (soil P); A × B; A × C; B × C; A × B × C

Grain Zn Concentration: 2; 2; 3; 3; 5; 5; *n.s.*

Grain P Concentration: 0.01; 0.01; 0.01; *n.s.*; *n.s.*; 0.02; *n.s.*

Grain Mg Concentration: *n.s.*; <0.01; <0.01; *n.s.*; *n.s.*; 0.01; 0.01

Grain Cu Concentration: *n.s.*; 0.2; 0.3; *n.s.*; 0.6; 0.6; *n.s.*

Grain Mn Concentration: 2; 2; 3; *n.s.*; 5; 5; 7

enhanced by higher P supply and unaffected by Zn supply. Autoclaving the soil reduced their grain concentrations markedly in the low-P treatment. Finally, all treatments had prominent effects on the grain Mn concentration. Higher Zn supply decreased the grain Mn whereas soil autoclaving and higher P application increased it significantly.

The Zn–P interaction was also studied in solution culture with varied Zn and P supply. In the low-Zn treatment, the plants appeared smaller at higher P levels and showed more severe leaf symptoms including chlorosis and necrosis (Fig. 3). The medium P level increased the shoot dry weight of the low-Zn plants when compared to the low P level, but a further increase in P

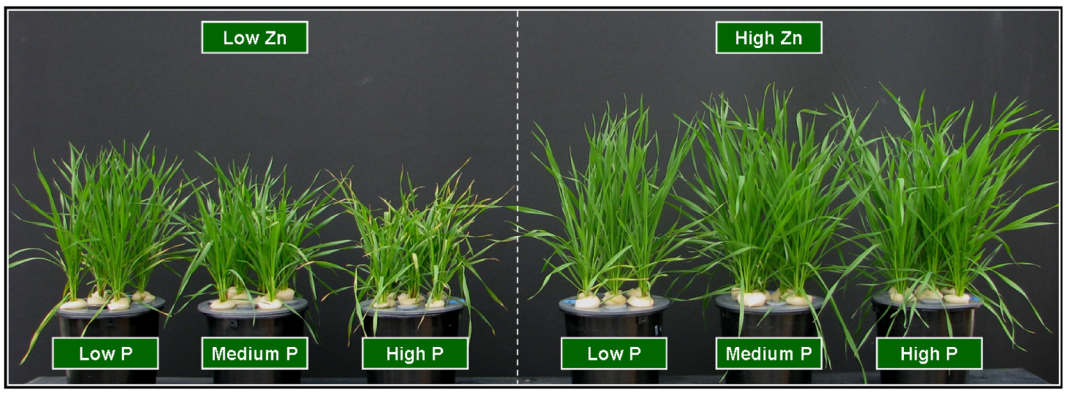

Fig. 3 34-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants grown hydroponically at different Zn (low: 0.01 μM; high: 1 μM) and P (low: 20 μM; medium: 100 μM; high: 500 μM) levels under greenhouse conditions

supply tended to decrease the dry shoot biomass (Table 4). In contrast, at the medium and high Zn levels, the plants produced significantly more shoot biomass with the medium or high P supply than with the low P supply (Fig. 3; Table 4). In terms of root dry weight, both lower Zn and lower P supplies were associated with higher values. Consequently, the root-to-shoot ratio increased dramatically with decreasing Zn and/or P availability. The root-to-shoot ratio of the low-Zn-low-P plants was over three times as high as that of the high-Zn-high-P plants.

In this solution culture experiment, the shoot Zn concentration increased greatly with increasing Zn supply, but did not vary according to the P supply (Table 5). The shoot P concentrations were elevated significantly not only by higher P supplies but also by the low Zn level. The extent of the "low-Zn effect" on the shoot P concentration depended on the P supply and was most prominent at high P. At low Zn, the shoot Mg and Cu concentrations were not affected by the P level and significantly higher than at higher Zn levels. However, at medium and high Zn, the low-P treatment was associated with markedly lower Mg and Cu concentrations in shoots as compared to the medium-P and high-P treatments. Significantly elevated Mn concentrations were measured in shoots under both the low-Zn and low-P conditions.

In the roots of hydroponically grown wheat plants, the Zn concentration was almost tripled with each increase in Zn supply, and the P concentration was doubled with each increase in P supply (Table 6). The Zn and P treatments did not have any effects on the root concentrations of each other. Significantly reduced Mg concentrations were measured in the roots of both the low-Zn and low-P plants. The low-P treatment also lowered the root Cu concentration. However, in contrast to Mg, Cu accumulated to higher concentrations in the roots of the low-Zn plants as compared to the medium-Zn and high-Zn plants. Increasing P supply had a lowering effect on the root Mn concentrations in all Zn treatments, and this effect was particularly pronounced in the high-Zn treatment.

In the next solution culture experiment, which was conducted to study ^{70}Zn uptake, the shoot and root dry weights of 20-day-old wheat plants were affected by only the P treatment (Table 7). The shoot dry weights exhibited a tendency to increase with increasing P supply whereas the root dry weights decreased slightly. Consequently, the low-P plants had greater root-to-shoot ratios than the medium-P and high-P plants. As shown in Fig. 4a, the cumulative ^{70}Zn uptake per unit root biomass values obtained for the low-Zn plants were significantly higher than those obtained for the high-Zn plants at all time points during the 7-h uptake experiment. In the low-Zn group, the low-P plants absorbed

Table 4 Shoot and root dry weights (DW) and root/shoot ratios of hydroponically-grown 34-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants as affected by Zn (low: 0.01 μM; medium: 0.1 μM; high: 1 μM) and P (low: 20 μM; medium: 100 μM; high: 500 μM) concentrations under greenhouse conditions

Solution Zn (A)	Solution P (B) (mg plant^{-1})	Shoot DW (mg plant^{-1})	Root DW (%)	Root/shoot
Low	Low	147±2	110±5	77±3
	Medium	207±30	84±14	42±14
	High	174±19	85±15	50±14
Medium	Low	210±8	94±5	45±1
	Medium	278±9	73±7	26±2
	High	284±12	64±5	23±1
High	Low	217±7	103±6	48±2
	Medium	270±26	63±19	23±6
	High	296±13	67±11	23±4

Values are means and standard deviations of 4 pot replicates (20 plants per pot)

$HSD_{0.05}$ values: A (solution Zn); B (solution P); A × B

Shoot DW: 18; 18; 43

Root DW: 11; 11; *n.s.*

Root/Shoot: 7; 7; *n.s.*

Table 5 Shoot Zn, P, Mg, Cu and Mn concentrations of hydroponically-grown 34-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants as affected by Zn (low: 0.01 μM; medium: 0.1 μM; high: 1 μM) and P (low: 20 μM; medium: 100 μM; high: 500 μM) concentrations under greenhouse conditions

Solution Zn (A)	Solution P (B)	Shoot mineral concentrations				
		Zn (mg kg^{-1})	P (%)	Mg (%)	Cu (mg kg^{-1})	Mn (mg kg^{-1})
Low	Low	5.6±0.4	0.25±0.00	0.29±0.01	12.1±1.6	222±5
	Medium	5.5±0.5	0.74±0.07	0.29±0.04	10.1±1.3	163±19
	High	6.5±0.8	2.13±0.17	0.32±0.05	10.9±0.4	173±23
Medium	Low	21.9±2.3	0.19±0.01	0.14±0.02	7.2±0.5	134±17
	Medium	19.3±1.2	0.52±0.04	0.20±0.01	10.1±0.9	124±8
	High	19.1±1.1	0.83±0.05	0.19±0.02	9.4±0.8	111±9
High	Low	48.1±0.9	0.18±0.01	0.14±0.01	7.7±0.4	121±11
	Medium	46.1±4.6	0.51±0.02	0.19±0.02	9.3±0.7	106±9
	High	49.2±6.1	0.83±0.04	0.18±0.02	10.1±1.1	104±9

Values are means and standard deviations of 4 pot replicates (20 plants per pot)

$HSD_{0.05}$ values: A (solution Zn); B (solution P); A × B

Shoot Zn Concentration: 2.9; *n.s.*; *n.s.*

Shoot P Concentration: 0.07; 0.07; 0.17

Shoot Mg Concentration: 0.03; 0.03; *n.s.*

Shoot Cu Concentration: 0.9; 0.9; 2.3

Shoot Mn Concentration: 14; 14; 35

Table 6 Root Zn, P, Mg, Cu and Mn concentrations of hydroponically-grown 34-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants as affected by Zn (low: 0.01 μM; medium: 0.1 μM; high: 1 μM) and P (low: 20 μM; medium: 100 μM; high: 500 μM) concentrations under greenhouse conditions

Solution Zn (A)	Solution P (B)	Root mineral concentrations				
		Zn (mg kg^{-1})	P (%)	Mg (%)	Cu (mg kg^{-1})	Mn (mg kg^{-1})
Low	Low	5.7±0.2	0.16±0.00	0.13±0.02	28.0±4.7	96±13
	Medium	7.5±0.9	0.37±0.09	0.12±0.02	47.3±9.7	89±20
	High	8.4±0.3	0.71±0.06	0.15±0.03	45.0±8.7	78±6
Medium	Low	21.8±2.3	0.18±0.01	0.16±0.01	21.3±1.6	115±11
	Medium	18.1±1.2	0.40±0.01	0.29±0.01	24.3±3.2	89±8
	High	19.9±2.6	0.80±0.16	0.25±0.05	23.3±3.7	73±10
High	Low	49.2±3.2	0.16±0.01	0.15±0.02	17.2±1.6	107±14
	Medium	48.6±3.1	0.44±0.10	0.29±0.04	23.8±5.4	69±7
	High	51.1±5.2	0.80±0.15	0.29±0.03	27.1±4.7	39±3

Values are means and standard deviations of 4 pot replicates (20 plants per pot)

$HSD_{0.05}$ values: A (solution Zn); B (solution P); A × B

Root Zn Concentration: 2.8; *n.s.*; *n.s.*

Root P Concentration: *n.s.*; 0.09; *n.s.*

Root Mg Concentration: 0.03; 0.03; 0.07

Root Cu Concentration: 5.6; 5.6; 13.1

Root Mn Concentration: 11; 11; 27

Table 7 Shoot and root dry weights (DW) and root/shoot ratios of hydroponically-grown 20-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants as affected by Zn (low: 0.01 μM; high: 1 μM) and P (low: 20 μM; medium: 100 μM; high: 500 μM) concentrations under greenhouse conditions (^{70}Zn uptake experiment)

Solution Zn (A)	Solution P (B)	Shoot DW (mg plant^{-1})	Root DW (mg plant^{-1})	Root/shoot (%)
Low	Low	103±4	73±5	71±6
	Medium	114±10	69±8	60±2
	High	110±8	62±8	56±7
High	Low	101±6	72±6	71±3
	Medium	113±11	65±5	58±2
	High	105±5	61±3	58±3

Values are means and standard deviations of 4 pot replicates (8 plants per pot)

$HSD_{0.05}$ values: A (solution Zn); B (solution P); A × B

Shoot DW: *n.s.*; 10; *n.s.*

Root DW: *n.s.*; 8; *n.s.*

Root/shoot: *n.s.*; 6; *n.s.*

only about half as much ^{70}Zn as the medium-P and high-P plants. The same negative effect of the low-P treatment on the ^{70}Zn uptake per unit root biomass was also observed in the high-Zn treatment as a trend though the differences were statistically not significant. One day after the end of the uptake experiment, both the shoot and the root ^{70}Zn concentrations measured in the low-Zn plants were markedly higher than those measured in the high-Zn plants (Fig. 4b, c). While the ^{70}Zn concentrations in the high-Zn plants were unaffected by the P nutritional status, higher P supplies were associated with significantly higher ^{70}Zn concentrations in the low-Zn plants.

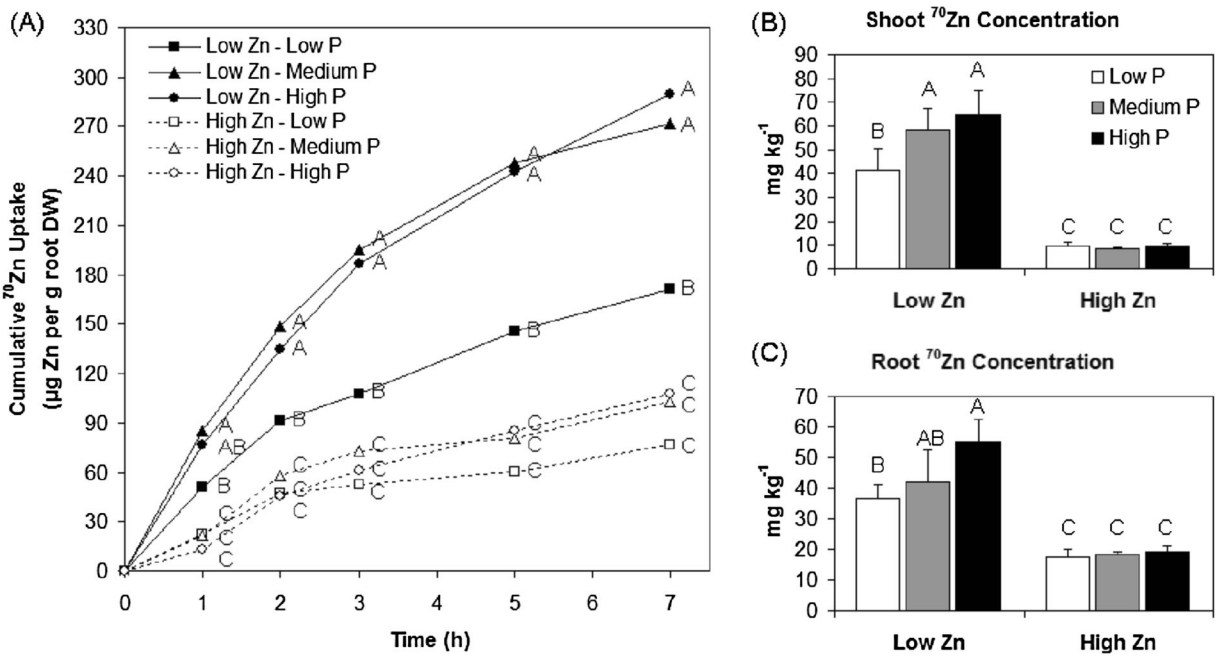

Fig. 4 **a** Cumulative ^{70}Zn uptake of 19-day-old, and **b** shoot and **c** root ^{70}Zn concentrations of 20-day-old bread wheat (*Triticum aestivum* cv. Adana99) plants grown hydroponically in a growth chamber at different Zn (low: 0.01 μM; high: 1 μM) and P (low: 20 μM; medium: 100 μM; high: 500 μM) levels. *Different letters* indicate significantly different means according to Tukey's HSD test ($p<0.05$)

Discussion

In the soil experiment, the positive responses of the shoot dry weight to higher Zn and P applications show that both nutrients were limiting plant growth at low levels under given growth conditions (Table 1). Although the interaction of Zn and P did not significantly affect the shoot dry weight, the grain yield showed a significant response to this interaction. The lack of a yield response to higher Zn application at low P indicates that P was the primary limiting nutrient under this condition. Clear responses to Zn were observed at higher P levels, where Zn and P applications augmented the effects of each other on grain yield. The P–Zn interaction had also some remarkable effects on the general condition of developing plants, particularly in non-autoclaved soil (Fig. 1a). In wheat, the typical leaf symptoms of Zn deficiency are whitish necrotic patches observed on middle-aged leaves (Cakmak et al. 1997; Sharma et al. 2004; Kutman et al. 2010). Moreover, Zn deficiency is often associated with delayed development in cereals (Cakmak et al. 1997; Neue et al. 1998; Genc et al. 2002). At low Zn, higher P supply aggravated these symptoms, indicating P-induced Zn deficiency. The clearer expression of this interaction in native soil indicates mycorrhizal involvement (Fig. 1).

Soil sterilization by autoclaving or other techniques soil can stimulate plant growth significantly, possibly by eliminating soil-borne pathogens and competing microorganisms (Meredith and Anderson 1992; Endlweber and Scheu 2006; Mahmood et al. 2014), unless it causes Mn toxicity by killing the Mn-oxidizing bacteria that transform plant-available Mn^{2+} into unavailable higher oxides (Boyd 1971; Williams-Linera and Ewel 1984). Here, irrespective of the Zn and P treatments, autoclaving had a modest positive effect on shoot growth but no significant effect on yield (Table 1), indicating that the non-autoclaved soil was not a significant source of biotic stress.

As a rule, applications of P fertilizers reduce mycorrhizal colonization of plant roots (Jackson et al. 2002; Ryan et al. 2008; Marschner 2012) although at extremely P-deficient conditions, low levels of P application may also have a positive impact (Bolan et al. 1984). The effects of Zn additions to soil on mycorrhizal colonization of roots can be negative, positive or neutral, depending on the conditions (Cavagnaro 2008). In many studies dealing with non-toxic levels of Zn fertilizers, the mycorrhizal colonization was unaffected by Zn treatments (McIlveen and Cole 1979; Ortas et al. 2002; Subramanian et al. 2009). Here, in agreement with the literature, the mycorrhizal colonization in non-autoclaved soil was drastically suppressed by higher levels of P but unaffected by Zn fertilization (Fig. 2), which was necessary for preventing Zn deficiency (Fig. 1) and maximizing grain yield (Table 1). It is also well-documented that sterilization of soil by autoclaving eliminates native AM (Smith and Smith 1981; Endlweber and Scheu 2006). Therefore, any indirect effects of P fertilization mediated by changes in mycorrhizal activity should disappear in autoclaved soil.

In the soil study, the shoot P concentrations measured in 81-day-old plants supplied with low P were below the P adequacy range, which is reported as ~0.2–0.5 % dry weight for wheat at this developmental stage (Table 2; Reuter and Robinson 1997). It is well established that AM can significantly contribute to P uptake of plants, especially under P-deficient conditions (Smith and Read 2008; Marschner 2012). The marked decrease in the shoot P concentrations of the low-P plants as a result of soil autoclaving can be explained by the elimination of mycorrhizal activity (Fig. 2; Table 2). Dilution effect on its own cannot account for this decrease because the plants grown in non-autoclaved soil had a 50 % higher shoot P concentration but only a 13–19 % lower shoot biomass than those grown in autoclaved soil (Tables 1 and 2). The apparent benefit of AM on the grain P levels of the low-P plants were even greater since the grain yields of mycorrhizal plants were at least as high as the plants grown in sterilized soil and thus, dilution effect was out of question (Tables 1 and 3). Reportedly, the AM contribution to P uptake decreases with increasing soil P supply and direct root uptake becomes more important (Nagy et al. 2009). In agreement, the effect of soil sterilization on the shoot and grain P concentrations started to fade at the medium P and disappeared at the high P supply (Tables 2 and 3).

Wheat is considered Zn-deficient if the shoot Zn concentration is lower than 10–15 mg/kg dry weight (Dang et al. 1993; Cakmak et al. 1996; Reuter and Robinson 1997). Accordingly, in the soil experiment, all plants grown at low Zn were either marginally low in Zn or Zn-deficient whereas all grown at high Zn were Zn-sufficient (Table 2). At low P, where the mycorrhizal activity is particularly high in non-autoclaved soil (Fig. 2), soil autoclaving dramatically reduced both shoot and grain Zn concentrations (Tables 2 and 3). The diminishing effect of soil autoclaving on tissue Zn

concentrations at higher P levels points out the critical role of AM in Zn uptake (Table 2 and 3). It is well documented that AM colonization of roots enhances plant Zn uptake under non-toxic conditions (Kothari et al. 1991; Ryan and Angus 2003; Watts-Williams et al. 2013). In non-autoclaved soil, the P-induced decreases in tissue Zn concentrations were remarkable at both the low and high Zn supply. Consideration of the large biomass and yield responses to higher P applications (Table 1) might lead to the deception that dilution was responsible for this effect. However, although similar biomass responses to higher P applications were observed in both non-autoclaved and autoclaved soil conditions, the negative effect of higher P on shoot and grain Zn concentrations was significantly weaker (at low Zn) or totally absent (at high Zn) in autoclaved soil (Tables 1, 2, and 3). Thus, in agreement with several previous studies on cereals (Li et al. 2003; Ryan et al. 2008; Zhang et al. 2012), the P–Zn interaction cannot be simply explained by dilution. These observations provide strong evidence that the P effects on tissue Zn concentrations of soil-grown plants are mediated primarily by AM. This may also explain why P-induced Zn deficiency was not observed in soil-grown canola, which is a non-mycorrhizal species (Lu et al. 1998).

In the solution culture experiment, both low Zn and low P impaired the shoot growth, stimulated the root growth, and thereby led to markedly elevated root-to-shoot ratios (Table 4) in accordance with the literature (Anuradha and Narayanan 1991; Cakmak et al. 1994; Erenoglu et al. 2011). High P supply visibly worsened the general condition of the low-Zn plants in solution culture (Fig. 3) as it did also in non-autoclaved soil culture (Fig. 1). However, the physiological reasons behind this apparently similar effect were different in these two systems. In non-autoclaved soil, higher P significantly lowered tissue Zn concentrations (Tables 2 and 3) most probably by suppressing AM (Fig. 2) whereas in AM-free solution culture, in agreement with some previous reports (Cakmak and Marschner 1986; Nichols et al. 2012), higher P did not decrease shoot or root Zn concentrations at any Zn level (Tables 5 and 6). Instead, Zn-deficiency-induced P toxicity was responsible for the deteriorated condition of the plants at low Zn and high P in this solution culture experiment (Fig. 3; Table 5). Here, the shoot P concentration exceeded 2 % dry weight, which is well above the P toxicity thresholds reported in the literature (Loneragan et al. 1982; Reuter and Robinson 1997). The excess P in the tissues may, in addition to causing direct toxicity, also have decreased the physiological availability of Zn (Cakmak and Marschner 1987). In the soil study, P toxicity was not observed in Zn-deficient plants grown with high P (Table 2), presumably because plant-available P in the alkaline and calcareous soil used in this study was never as abundant as it was in the nutrient solution.

It was very obvious that the level of P supply had a marked effect on the tissue Mg concentrations in both the soil and solution culture experiments (Tables 2, 3, 5, and 6). Phosphorus deficiency reduced the shoot Mg concentrations to ~0.14 % dry weight (Tables 2 and 5),

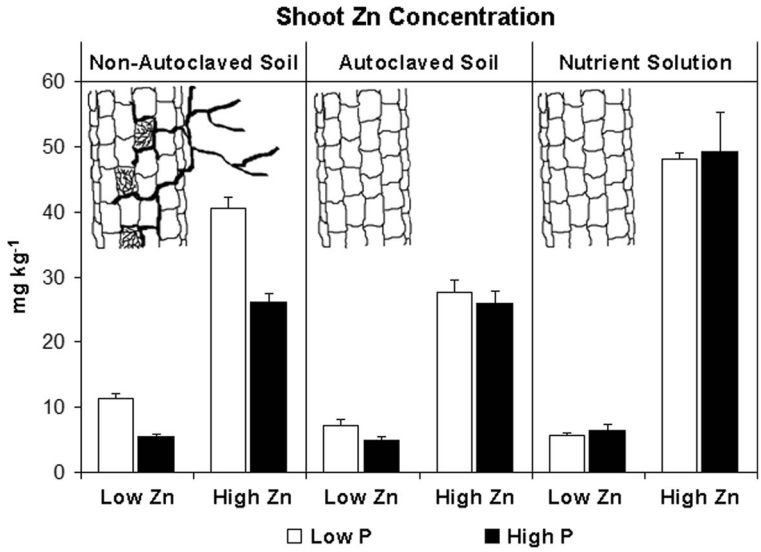

Fig. 5 Effects of P supply on the shoot Zn concentrations of bread wheat plants (*Triticum aestivum* cv. Adana99) grown in non-autoclaved soil (with natural mycorrhiza), autoclaved soil (without mycorrhiza) and nutrient solution (without mycorrhiza) under low-Zn and high-Zn conditions (values were compiled from Tables 2 and 5)

which is the critical level for Mg deficiency in wheat (Reuter and Robinson 1997). That P deficiency could induce Mg deficiency and P fertilization enhanced tissue Mg concentrations was documented in several field and solution culture studies on various species including grapevine (*Vitis vinifera*), squash (*Cucurbita pepo*), tall fescue (*Festuca arundinacea*) and wheat (Skinner and Matthews 1990; Reinbott and Blevis 1994, 1999). As mycorrhizal colonization of roots can contribute significantly to Cu uptake of plants (Kothari et al. 1991; Lambert and Weidensaul 1991; Li et al. 1991), Cu can be expected to respond to the sterilization and P treatments, which affect mycorrhizal activity in the soil study. Accordingly, the reduction of the grain Cu concentration by soil sterilization at low P (Table 3) may be due to the elimination of mycorrhizal activity. However, tissue Cu concentrations were in many cases not reduced but enhanced by higher P supply (Tables 2, 3, 5, and 6), suggesting that factors other than AM are involved in the effects of P supply on Cu in wheat.

Arbuscular mycorrhiza is known to attenuate Mn uptake of plants by stimulating Mn-oxidizing and suppressing Mn-reducing bacteria and thus lowering the bioavailability of Mn in the soil (Kothari et al. 1991; Arines et al. 1992; Ryan and Angus 2003; Nogueira et al. 2004). This, in addition to the 5-fold increase in the DTPA-extractable Mn concentration of the soil upon autoclaving (see Materials and methods for details), explains why shoot Mn concentrations were significantly enhanced by soil sterilization as well as higher P supply in non-autoclaved soil (Table 2). In contrast, the shoot and root Mn concentrations decreased with increasing P supply in solution culture (Tables 5 and 6), probably due to dilution (Table 4). The positive response of the grain Mn concentration to higher P supply, which is observed in not only non-autoclaved but also sterilized soil (Table 3), may be related to the possible role of phytate as a storage compound for Mn in wheat grain (Rodrigues-Filho et al. 2005).

The effect of P supply on ^{70}Zn uptake of wheat was studied on young, hydroponically-grown plants, which were not significantly different from each other with respect to biomass but just starting to show growth responses to Zn and P treatments (Table 7), in order the minimize the potential impact of growth differences on mineral uptake. Zinc starvation was reported to increase Zn uptake rates in wheat by de-repression of the uptake system (Rengel et al. 1998; Erenoglu et al. 2011). Accordingly, plants pre-cultured with low Zn absorbed remarkably higher levels of ^{70}Zn than plants pre-cultured with high Zn (Fig. 4). Most importantly, low-P plants were inefficient in ^{70}Zn uptake when compared to P-sufficient plants. This means that in the absence of AM, P-deficiency may impair direct Zn uptake by plant roots.

The main findings of this study are summarized in Fig. 5. In non-autoclaved soil, higher P supply reduces tissue Zn concentrations of wheat in both Zn-deficient and Zn-sufficient conditions, primarily by suppressing mycorrhizal activity. This effect disappears in autoclaved soil, where AM fungi have been eliminated. Solution culture conditions are in this respect representative for autoclaved soil. All these results indicate that the well-documented negative effect of high P on tissue Zn concentration is mycorrhiza-dependent. By avoiding excessive P fertilization and implementing AM-stimulating agronomic practices, P-induced losses to Zn concentrations may be minimized. The AM-mediated P–Zn interaction in wheat should also be considered in biofortification of cereal grains with Zn.

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