Effect of zinc foliar application and mycorrhizal inoculation on morpho-physiological traits and yield parameters of two barley cultivars

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Abstract

Zinc (Zn) plays a vital role in biological systems. Plants require an appropriate balance of this essential micronutrient for growth and optimum yield. This study focused on the effectiveness of foliar application of Zn combined with inoculation with arbuscular mycorrhizal (AM) fungi on morphological, physiological traits and yield parameters of barley cultivars during the 2015-2016 growing season. In this factorial experiment, different forms of foliar applied ZnO (nil, nano Zn, ordinary Zn and nano+ordinary Zn) and inoculation with AM fungi (nil, *Glomus mosseae* and *Rhizophagus irregularis*) were investigated for two barley cultivars (Yusuf and Julgeh). The two cultivars differed in response to the form of foliar Zn applied and inoculation with the two commercial inocula of AM fungi. The major responses were significant increases in chlorophyll content (107%), soluble sugar (227%), grain Zn concentration (217%), carbonic anhydrase activity (128%) and grain phytase activity (65%) for cultivar Julgeh inoculated with *G. mosseae* when sprayed with nano ZnO compared with control. Cultivar Julgeh inoculated with *G. mosseae* had physiological traits more likely to enhance productivity and economical yield than did cultivar Yusuf that invested more in root traits and vegetative growth. Consequently, the nano form of Zn positively increased root and shoot morphological parameters, physiological parameters and grain Zn concentration, but the ordinary form of Zn enhanced yields and yield parameters. While foliar Zn application and inoculation with AM fungi significantly enhanced all measured parameters, the forms of Zn and inoculation with the two different AM fungi differed in their effectiveness.

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Introduction

Zinc (Zn) is a crucial micronutrient that plays a principal role in growth and development of plants and animals as a co-factor for more than 300 enzymes involved in the metabolism of carbohydrates, lipids, proteins, and nucleic acids (Sadeghzadeh, 2013). It is estimated that construction of about 3000 proteins in the human body is dependent on the presence of Zn (Andreini *et al*., 2006). The main reasons for Zn deficiency in biological systems are the lack of this nutrient in the soil (*e.g*. in Zn-deficient soil), its limited solubility in soil and low levels in grain (Fileppi *et al*., 2010). Many physiological perturbations resulting from Zn deficiency are associated with disruption of enzyme activity, thus Zn-deficiency induced inhibition of photosynthesis is coincident with a decrease in activity of key photosynthetic enzymes (Brown *et al*., 1993). Carbonic anhydrase, which is present in all photosynthetic tissues and is required for chlorophyll biosynthesis, has a requirement for Zn (Rehman *et al*., 2012). Therefore, Zn deficiency can reduce rate of photosynthesis due to a sharp decline in carbonic anhydrase activity (Barman *et al.,* 2018). However, Zn efficient crop genotypes with high ability to mobilisation, uptake, utilisation and translocation of Zn under low Zn availability (Chaab *et al*., 2011) have potential to produce more dry matter and grain yield by absorbing more Zn from Zn-deficient soils (Sundaram and Stalin, 2016). Cultivation of high yielding wheat and barley can lead to a gradual increase in nutrient deficiency, including Zn deficiency, in plants with a direct negative impact on quality and quantity of crop production (Cakmak, 2009). Hence, there is a need to identify Zn efficient crop genotypes with high Zn utilisation efficiency (Singh and Singh, 2011). Interactions between Zn and other nutrients in soil, especially phosphorus (P), can also increase Zn deficiency in soil (Imran *et al*., 2016) and during transmission from root to shoot (Zhang *et al*., 2016). In a study by Zhang *et al*. (2012a), P applications greater than 200 kg ha^{-1} resulted in significantly increased P content in different wheat tissues, although the concentration of Zn in wheat grain was reduced. To address the reduction in grain Zn concentration for high levels of P application, Zhang *et al*. (2012a) recommended foliar Zn application to reduce the P/Zn molar ratio in wheat and consequently increase Zn bioavailability.

Foliar application of Zn has potential to increase Zn concentration in wheat grain without soil interactions restricting Zn availability (Velu *et al*., 2014; Deshpande *et al*., 2018) because of its greater efficiency in grain Zn accumulation in comparison to soil application (Jan *et al*. 2016). It is a strategy for providing adequate Zn nutrition in wheat because Zn is highly mobile in phloem and can be effectively transported from leaves and stems to developing grain (Haslett *et al*., 2001). Zhang *et al*. (2012b) showed that grain Zn concentrations in wheat were increased by 73% with foliar application of $ZnSO_4$. Similarly, foliar Zn application was more effective for increasing Zn concentrations in maize, rice and wheat grain compared to soil application of Zn (Joy *et al*., 2015). It could also be effective for barley in curbing element imbalance, especially in semi-arid areas where the intensive crop production are annually exposed to high applications of P fertilisers with potential micronutrient deficiency in grain (Jalali, 2007).

The use of nano fertilisers in foliar applications is an emerging technology with potential benefits compared to soil-applied Zn fertilisers because of more rapid Zn absorption and reduced Zn leaching (Morales-Diaz *et al*., 2017). In a recent study on barley (Janmohammadi *et al*., 2016) some priorities for increasing crop production using nano-micronutrient fertilisers included their high efficiency, easy application and greater convenience. Nano fertilisers have potential to elevate solubility and mobility of poorly soluble nutrients in soils and increase their bioavailability (Naderi *et al*., 2013) due to the fact that they can enable nutrients to diffuse more slowly than ordinary fertilisers (Janmohammadi *et al*., 2016) with increased nutrient use efficiency (Wang *et al*., 2013).

Arbuscular mycorrhizal (AM) fungi can have a positive influence on plant physiology as well as on root architecture (Dhawal *et al*., 2016). AM fungi can enhance P and Zn absorption by host roots (Thompson *et al*., 2013) but application of P fertiliser can reduce Zn uptake associated with a reduction of mycorrhizal colonisation of roots (Teng *et al*., 2013). Increasing the availability of P in soil can negatively affect mycorrhizal colonisation, root Zn uptake and concentrations of Zn in wheat tissues (Ova *et al*., 2015). According to Nouri *et al*. (2014), the access to Pi is a systemic affinity that depends on the nutritional status of their host plant. AM fungi supply the host with various mineral nutrients in exchange for host assimilates so that the interruption in this symbiosis by high Pi levels, can be attributed as energy saving process for host plant without the loss of assimilates (Smith *et al*., 2009). Although this symbiotic limitation may occur at the expense of absorption of other elements (Blanke *et al*., 2005), a meta-analysis of field studies of inoculation of wheat with AM fungi can be an effective agronomic practice (Pellegrino *et al*., 2015).

Clearly, several factors can combine to influence the Zn concentration in grain. While there is potential for Zn and P fertilisers to interact and reduce availability of Zn in soil, foliar application of Zn fertiliser can avoid this problem. Furthermore, the alleviation of potential interactions between Zn and P in soil by foliar application of Zn can be complemented by colonisation of roots by AM fungi to support effective use of P fertiliser. Therefore, the combined effects of foliar application of different forms of Zn and inoculation with commercial inocula of AM fungi were investigated for two barley cultivars. The hypotheses were: i) that the nano form of ZnO would be more effective at increasing Zn concentration in barley grain than the ordinary form of ZnO, due to its smaller size and ease of uptake from foliar application; ii) that barley cultivars would differ in response to both the form of Zn applied to barley leaves and commercial inocula of AM fungi due to their diverse performance and variation in physiological, phenotypical traits; and iii) that there could be an interaction between the form of foliar Zn application and effect of AM fungal inoculation for the two barley cultivars studied here.

Materials and methods

Experimental design and planting

The experiment was a factorial randomised complete design of two barley (*Hordeum vulgare* L.) cultivars (Yusuf and Julgeh), three AM fungal treatments and four forms of Zn application; there were three replicates of each treatment. It was conducted in pots placed under field conditions at the Agricultural and Governmental Research Centre of Chenaran, Iran (36°61' N, 59°16' E, altitude 1221 m) during the 2015-2016 cropping season. To make growing conditions much more similar to natural field conditions, the pots were placed in a field plot.

The three AM fungal treatments were: inoculation with commercial inocula of either *Glomus mosseae* or *Rhizophagus irregularis* and a control without inoculation. The commercial inocula of AM fungi were obtained from Turan Biotech, Shahrood. The inoculum consisted of a mixture of spores containing soil (with a density of 150 in 100 g of dry clay). The four foliar Zn oxide (ZnO) applications were: a control (NO Zn), Nano Zn (2 g L^{-1}), Ordinary Zn (2 g L^{-1}) and Nano Zn (1 g L^{-1}) + Ordinary Zn (1 g L^{-1})]. Both the ordinary ZnO particles with a larger diameter (average 200 nm) and nano Zn particles with a smaller diameter (average 20 nm) were obtained from Pioneer Nanomaterials Company of Iran.

Plastic pots (30 cm top diameter × 30 cm height) were filled with 10 kg of field soil in a mixture of sand (3:1, v:v). Soil was collected from a research farm located at the Agricultural and Governmental Research Centre of Chenaran, Iran. The soil was classified as sandy loam with the following characteristics: pH 7.8, organic matter 1.11%, Zn 0.11 ppm, P 70 ppm, Fe 2.1 ppm, K 550 ppm, Cu 0.5 ppm. Barley seeds were provided by the Centre of Agriculture and Natural Resources of Mashhad, Iran. Inoculum of AM fungi was applied at the time of sowing. The commercial mycorrhizal inocula was used according to the methods recommended by the company. After creating grooves of approximately 10 cm in depth within pots, a 2 cm layer of inoculum (10 g) was placed in the grooves manually and covered with two centimetres of soil. Six surface sterilised seeds pet pot were placed in the appropriate place on the soil and covered with four centimetres of soil.

Seedlings were thinned to four following emergence. Pots were placed in the field to approximate field growing conditions, with temporarily use of a rain shade in winter to protect against hail damage. All pots were subjected to natural solar radiation and irrigated to 100% soil water capacity (WC) (Paech and Simonis, 1952) daily by adding the required volume of water after weighing. To calculate 100% WC, the three pots were filled with soil and water were added till saturation. 6 hours were spent till water had drained by gravity and then weighted. After that soil was dried for two days in compartment dryer at 105°C and again weighted. Finally, 100% WC is calculated out of the weight of saturation soil minus the dry weight. Zn was applied to barley leaves using a hand sprayer until they were completely covered with the solution. Foliar spraying with the ZnO treatments was carried out twice; the first spray occurred at tillering and the second spray occurred when the grains were at milk stage. Sprays of the different forms of ZnO were applied at the same concentration of Zn while for the control (No Zn) treatment, pots were merely sprayed with water.

Physiological and yield measurements

All plants were harvested at physiological maturity (180 days after planting) and were removed from soil then shoots and roots were separated and dried at 70°C for 24 h. Shoot traits [plant height, flag leaf area, leaf area, leaf area:root area ratio, shoot dry weight, and leaf dry weight] and yield components [tiller number, spike number, number of grains plant^{-1}, thousand kernel weight, grain yield, harvest index, and straw yield] were recorded. For quantification of physiological parameters and enzyme activities in plants, three fresh leaves of each plant per replicate were collected two weeks after the second Zn application concurrent with physiological maturity at harvesting time. Chlorophyll content was assessed according to Porra *et al*. (1989), soluble sugar was assessed according to Yang *et al*. (2001), enzyme carbonic anhydrase was assessed according to the method of Gibson and Leece (1981), phytase activity was assessed according to Barrientos *et al*. (1994) after recording morphological parameters of harvested leaves. The concentration of Zn in grain was determined using the method of Chapman and Pratt (1961). To estimate Zn concentration in grain, all samples well washed and properly hulled in order to eliminate possible residential of Zn in the surface of grains. Grain samples were ground and digested with a boiling acid mixture (HNO_3 + $HClO_4$) then the concentrations of Zn in the digest were determined on an ARL 3520 inductively coupled plasma. To measure underground section, roots were thoroughly washed with tap water to remove soil prior to assessing the following root traits: total root length, root area, average root diameter, root volume, root depth, root dry weight, root:shoot dry weight ratio (R:S). Leaf area was measured using a Scanject Delta Scanner. Root parameters were measured using Delta T Scan Software (DELTA SCAN, England) and root images were analysed using WinRHIZO Pro software.

Data analysis

The experiment was set up in a completely randomised design with factorial arrangement of treatments and three replications. Data were analysed by one-way analysis of variance (ANOVA) using the statistical software SAS (version 9.1). The treatment mean values were compared by least significant difference test at 0.05 level of probability. Data on morphological and physiological traits and yield were subjected to a three-factor ANOVA (factorial 2×3×4). Pearson correlation coefficients were calculated for the five physiological traits, along with grain yield and yield parameters were implemented by using SAS software.

Results

Physiological responses

Chlorophyll, soluble sugar, carbonic anhydrase and grain phytase activity were all significantly influenced by treatments ($P<0.01$, Table 1). In both barley cultivars, physiological traits were markedly enhanced following inoculation with AM fungi and foliar Zn spraying (Figures 1 and 2). Physiological traits of cultivar Julgeh inoculated with *G. mosseae* and Yusuf inoculated with *R. irregularis* while both were sprayed with nano ZnO were significantly affected (Figures 1 and 2). Positive correlations between physiological traits carbonic anhydrase and grain phytase activity and the majority of yield parameters (tiller number, spike number, grain number, thousand kernel weight, harvest index and straw yield) were highly significant ($P<0.01$).

Zn in grain

Zn in grain was significantly affected by mycorrhizal inoculation and Zn spraying ($P<0.001$) and was significantly affected by the third order interactions between cultivar × mycorrhizal inoculation × Zn (Table 1). The concentration of Zn in barley grain was enhanced in both Yusuf and Julgeh following inoculation with AM fungi in combination with foliar Zn spraying compared with uninoculated plants and lack of Zn application, but this enhancement was greater in Julgeh than in Yusuf for the same treatments (Figure 2B). All forms of ZnO increased Zn concentration in grain with the most marked impact resulting from foliar spraying with nano ZnO (Figure 2B) compared to the ordinary form of Zn. Increasing the availability of Zn *via* foliar application or inoculation with AM fungi enhanced Zn supply to both barley cultivars.

Shoot and root responses

Foliar ZnO treatments and mycorrhizal inoculation significantly affected most shoot and root traits for both barley cultivars ($P<0.01$, Table 1). Although there were significant second and third order interactions among some traits, they were not signifi-

Table 1. Analysis of variance for root, shoot and physiological traits of two barley cultivars.

Traits	TRL (mm)	RA (mm^2)	ARD (mm)	RV (mm^3)	RD (cm)	RDW (g)	R/S	LA (cm^2)	LA/RA	SDW (g)	LDW (g)	Chl.T (mg^{-1}g fw)	SS (mg^{-1}g dw)	C.A (units.cm^2)	GPH (mg^{-1}units fw)	GZn (mg kg^{-1})
Cultivars (C)	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Mycorrhiza (M)	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Zinc (Z)	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
C × M	**	**	**	**	**	**	**	**	**	**	**	**	**	*	*	*
C × Z	**	ns	ns	**	ns	ns	ns	**	**	**	**	**	**	**	*	**
M × Z	**	*	*	**	**	*	**	**	**	**	**	**	**	**	ns	**
C × M × Z	**	ns	**	**	**	*	*	**	**	**	**	**	**	ns	ns	**
LSD	1888	4.27	0.001	0.0006	0.19	0.002	0.001	26.88	0.0009	0.001	0.0001	0.001	0.81	1367	0.01	0.21

TRL, total root length; RA, root area; ARD, average root diameter; RV, root volume; RD, root depth; RDW, root dry weight; R:S, root:shoot ratio; LA, leaf area; LA:RA, ratios of leaf area to root area; SDW, shoot dry weight; LDW, leaf dry weight; Chl. T, chlorophyll total; SS, soluble sugar; CA, carbonic anhydrase; GPH, grain phytase activity; GZn, grain zinc concentration; LSD, least significant difference. *Significant at $P<0.05$; **significant at $P<0.01$; ns, not significant.

cant for cultivar × Zn for root area, average root diameter, root depth, root dry weight and root to shoot ratio. Root and shoot morphological traits differed significantly between cultivars (Table 1) and had the lowest values in the absence of application of ZnO and AM fungi.

Application of nano Zn frequently showed higher values than other Zn forms for all root and shoot morphological traits as for the physiological parameters (Table 1). In general, for both barley cultivars, inoculation with AM fungi significantly increased all measured morphological traits of root and shoot (Table 1). Whereas inoculation with *R. irregularis* better illustrated symbiotic responses with cultivar Yusuf, inoculation with *G. mosseae* was more

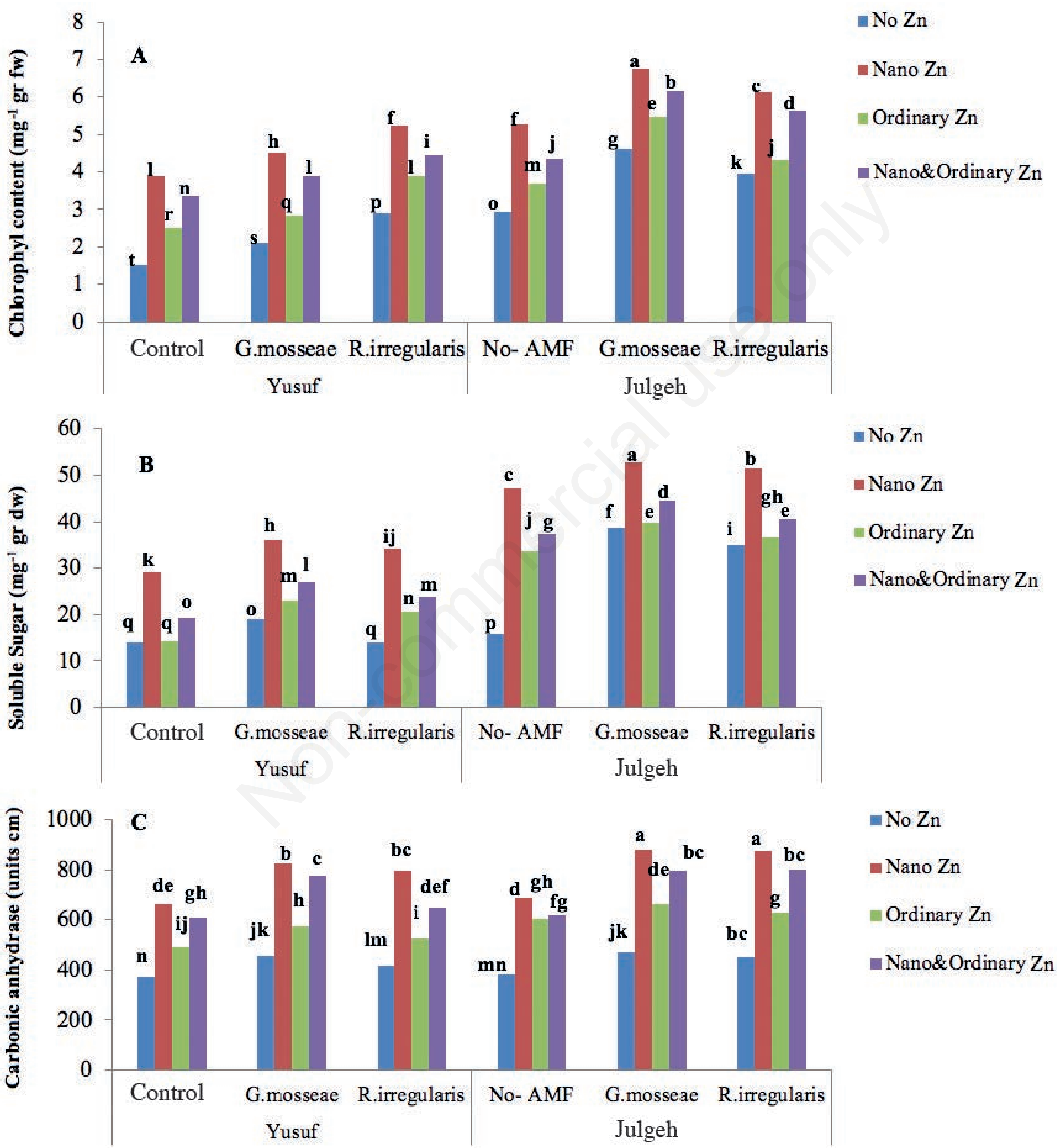

Figure 1. Effects of barley cultivars Yusuf and Julgeh, mycorrhizal inoculation and Zn foliar application on physiological traits chlorophyll total (A), soluble sugar (B) and carbonic anhydrase (C). Different letters represent significant differences (P<0.05).

effective with the host cultivar Julgeh.

Application of foliar Zn spray and inoculation with AM fungi significantly increased all yield components in both cultivars. Inoculation with *G. mosseae* significantly increased (63%) thousand kernel weight, grain yield (128%) and harvest index (61%) in cultivar Julgeh compared to non-inoculated Julgeh (Table 2). For cultivar Yusuf, inoculation with *R. irregularis* significantly increased tiller number (104%), plant height (41%) and straw yield (65%) in comparison with non-inoculated plants (Table 2). However, the effects on traits associated with yield were less consistent, since ordinary Zn was more effective than nano form to improve yield and yield components.

In addition, correlations between number of spike with grain phytase activity and grain Zn concentration were significant and positive ($r^2 \geq 0.90$), while grain Zn concentration was also correlated with tiller number, spike number, grain number, thousand kernel weight, harvest index and straw yield ($r^2 \geq 0.50$). There were positive and strong correlations between physiological traits and shoot characteristics ($r^2 \geq 0.50$) (Table 3). Soluble sugar was correlated with harvest index ($r^2 = 0.57$) whilst carbonic anhydrase was most strongly correlated with grain phytase activity, grain Zn and spike number ($r^2 \geq 0.90$, Table 3). There was also a correlation between grain yield and some yield components (Table 3).

Mycorrhizal inoculation resulted in stronger increases in all root morphological traits of both cultivars but these effects differed between cultivars. There were significant increases in root length (95%), root area (34%), root depth (61%), root dry weight (77%) and shoot dry weight (190%) in cultivar Yusuf when compared with the non-inoculated control (Table 4). For cultivar Julgeh, there were fewer significant effects of mycorrhizal inoculation compared with Yusuf, with increases in average root diameter and root volume of 100% and 211% respectively. For cultivar Julgeh, inoculation with *G. mosseae* and spraying with nano Zn increased leaf area by 18%, leaf area:root area 15% and leaf dry weight 27%

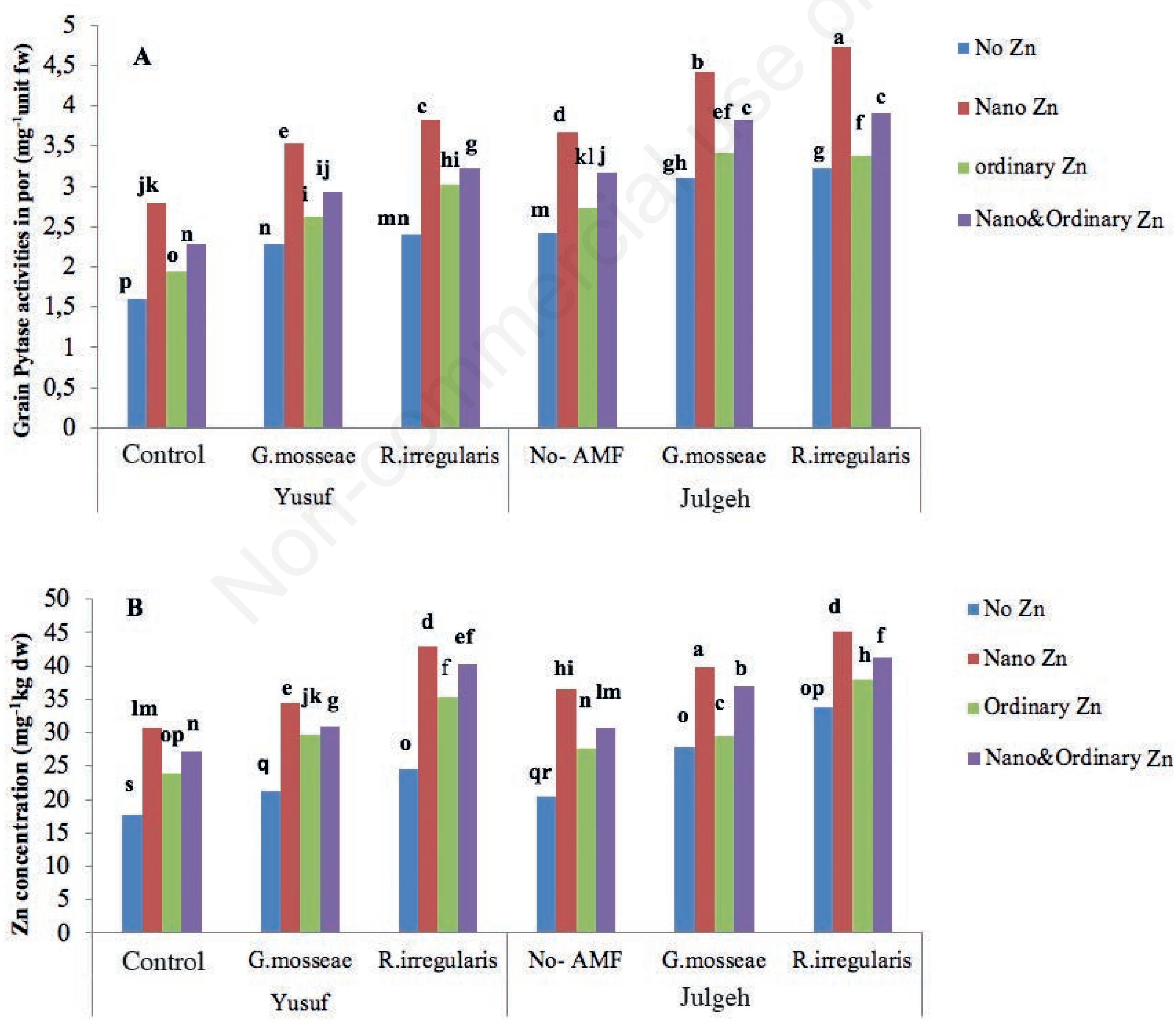

Figure 2. Effects of barley cultivars Yusuf and Julgeh, mycorrhizal inoculation and Zn foliar application on physiological traits phytase activity (A) and grain zinc concentration (B). Different letters represent significant differences ($P<0.05$).

compared with the non-inoculated and non-sprayed control. The highest root:shoot ratios for both cultivars were observed in the controls, which received neither foliar Zn sprays nor mycorrhizal inoculation. For cultivar Yusuf, there was a positive response in the majority of root morphological traits following inoculation with *R. irregularis* and spraying with nano Zn.

Discussion

In this study of two barley cultivars, it was shown that: i) application of Zn whether in the nano or ordinary forms increased the concentration of Zn in grain, and improved physiological traits and yield parameters; ii) inoculation with AM fungi strongly increased root and shoot morpho-physiological traits, Zn grain and yield of

Table 2. Effects of foliar Zn application and inoculation with mycorrhizal fungi on shoot and yield parameters of barley.

Cultivar	Mycorrhiza	Zn treatment	TN (no.)	SN (no.)	GN	FLA (cm^2)	PH (cm)	TKW (g)	GY ($g.pot^{-1}$)	HI (%)	SY ($g\ pot^{-1}$)
Yusuf	Control	Zn1	6.67^{m}	4.67^{p}	153.67^{v}	9.66^{u}	87.70^{n}	27.33^{n}	5.73^{u}	23.70^{s}	18.43^{jk}
		Zn2	7.67^{k}	5.67^{o}	168.67^{t}	11.84^{t}	91.30^{m}	33.10^{l}	7.63^{t}	27.18^{p}	20.43^{i}
		Zn3	11.67^{c}	9.67^{h}	182.67^{p}	21.25^{o}	105.1^{f}	38.10^{i}	10.6^{m}	34.11^{k}	20.53^{i}
		Zn4	9.67^{g}	7.67^{k}	174.67^{s}	17.31r	97.30^{j}	35.70^{j}	8.63^{r}	29.59^{n}	20.53^{i}
	G. mosseae	Zn1	7.67^{k}	6.67^{m}	159.67^{u}	18.24^{q}	92.70^{l}	31.70^{m}	8.03^{s}	26.18^{q}	22.63^{h}
		Zn2	9.67^{g}	8.67^{i}	174.67^{s}	21.06^{o}	98.50^{i}	37.63^{i}	9.13^{p}	28.04^{o}	23.43^{g}
		Zn3	12.67^{b}	11.67^{d}	188.67^{n}	32.83^{f}	116.1^{c}	42.63^{f}	12.63^{h}	33.10^{s}	25.53^{e}
		Zn4	10.67^{e}	9.67^{h}	180.67^{q}	25.90^{l}	106.9^{e}	40.03^{h}	10.83^{l}	30.80^{m}	24.33^{f}
	R. irregularis	Zn1	8.67^{i}	7.67^{k}	176.67^{r}	27.57^{j}	98.10^{i}	34.87^{k}	8.90^{q}	24.60^{r}	27.27^{d}
		Zn2	10.67^{e}	8.67^{i}	184.67^{o}	32.15^{g}	110.1^{d}	42.43^{f}	10.70^{m}	27.31^{p}	28.47^{c}
		Zn3	13.67^{a}	11.67^{d}	202.67^{l}	42.46^{b}	124.1^{a}	47.33^{cd}	13.70^{e}	31.01^{m}	30.47^{a}
		Zn4	11.67^{c}	10.67^{f}	195.67^{m}	36.76^{e}	117.1^{b}	44.13^{e}	12.00^{i}	29.14^{n}	29.17^{b}
Julgeh	Control	Zn1	4.00^{p}	6.00^{n}	242.67^{k}	15.14^{s}	73.43^{s}	32.83^{l}	7.70^{t}	37.75^{j}	12.67^{o}
		Zn2	5.00^{o}	8.00^{j}	268.67^{i}	19.23^{p}	77.03^{r}	37.37^{i}	9.30^{o}	42.88^{gh}	12.37^{o}
		Zn3	8.00^{j}	11.00^{e}	328.67^{k}	26.86^{k}	90.83^{m}	42.37^{f}	13.30^{f}	51.19^{b}	12.67^{o}
		Zn4	6.00^{n}	10.00^{g}	294.67^{i}	22.33^{n}	83.03^{p}	39.27^{h}	11.20^{k}	46.89^{e}	12.67^{o}
	G. mosseae	Zn1	8.00^{j}	8.00^{j}	284.67^{h}	23.96^{m}	83.83^{o}	40.83^{g}	11.57^{j}	43.17^{g}	16.30^{m}
		Zn2	9.00^{h}	10.00^{a}	328.67^{e}	29.71^{i}	95.83^{k}	47.63^{c}	13.07^{g}	47.17^{e}	17.60^{l}
		Zn3	11.00^{d}	14.00^{g}	384.67^{a}	47.42^{a}	109.8^{d}	53.63^{a}	17.57^{a}	60.90^{a}	18.10^{k}
		Zn4	10.00^{f}	12.00^{c}	346.67^{c}	38.92^{c}	102.8^{g}	50.63^{b}	15.07^{c}	50.65^{c}	18.60^{j}
	R. irregularis	Zn1	5.00	7.00^{l}	262.67^{j}	19.42^{p}	78.43^{q}	35.33^{jk}	10.27^{n}	41.47^{i}	13.50^{n}
		Zn2	7.00^{l}	8.00^{j}	297.67^{f}	23.62^{m}	84.23^{o}	42.67^{f}	12.07^{i}	42.58^{h}	13.50^{n}
		Zn3	9.00^{h}	13.00^{b}	378.67^{b}	38.44^{d}	101.8^{h}	50.67^{b}	16.37^{b}	49.23^{d}	10.50^{p}
		Zn4	7.00^{l}	10.00^{g}	335.67^{d}	30.80^{h}	92.63^{l}	46.67^{d}	13.87^{d}	44.73^{f}	13.50^{n}
		LSD (0.05)	1.3404	1.3404	5.9946	2.5069	1.6057	1.3623	1.2033	2.6403	0.905

In the same column, values marked with the same letters are similar ($P<0.05$), whereas those with different letters are significantly different. Zn1= no spraying (control); Zn2 = nano ZnO; Zn3= ordinary ZnO; Zn4 = nano + ordinary ZnO. TN, tiller number; SN, spike number; GN, number of grains per plant; FLA, flag leaf area; TKW, thousand kernel weight; GY, grain yield; HI, % harvest index; SY, straw yield; LSD, least significant difference.

Table 3. Correlations coefficients between physiological traits with yield and yield parameters.

	Chl.T ($mg\ g^{-1}$ fw)	SS ($mg\ g^{-1}$ dw)	C.A ($units.cm^2$)	GPH (Mg/units fw)	GZn ($mg\ kg^{-1}$)	TN (no.)	SN (no.)	GN	FLA (cm^2)	PH (cm)	TKW (g)	GY ($g\ pot^{-1}$)	HI (%)	SY ($g\ pot^{-1}$)
Ch.T	1.00	0.00	0.57**	0.61**	0.70**	0.18ns	0.53**	0.28**	–0.52**	0.24*	0.90**	0.16ns	0.41**	0.47**
SS		1.00	0.29**	0.28**	0.13ns	0.45**	0.17ns	0.35**	0.45**	0.56**	0.24*	0.47**	0.57**	0.51**
CA			1.00	0.96**	0.90**	0.80**	0.94**	0.83**	0.01ns	0.48**	0.66**	0.47**	0.70**	0.58**
GPH				1.00	0.91**	0.79**	0.92**	0.78**	–0.037ns	0.52**	0.70**	0.44**	0.68**	0.57**
GZn					1.00	0.72**	0.89**	0.72**	–0.20ns	0.38**	0.71**	0.27**	0.55**	0.50**
NT						1.00	0.80**	0.79**	0.20	0.47**	0.33**	0.06**	0.53**	0.42**
NS							1.00	0.89**	–0.03ns	0.44**	0.58**	0.42**	0.62**	0.52**
GN								1.00	0.18ns	0.50**	0.40**	0.49**	0.63**	0.51**
FLA									1.00	0.55**	–0.18ns	0.64**	0.46**	0.43**
PH										1.00	0.51**	0.76**	0.80**	0.82**
TKW											1.00	0.50**	0.71**	0.76**
GY												1.00	0.90**	0.88**
HI													1.00	0.93**
SY														1.00

Chl.T, chlorophyll total; SS, soluble sugar; CA, carbonic anhydrase; GPH, grain phytase activity; GZn, grain zinc concentration; TN, tiller number; SN, spike number; GN, number of grains per plant; FLA, flag leaf area; PH, plant height; TKW, thousand kernel weight; GY, grain yield; HI, % harvest index; SY, straw yield. *Significant at $P<0.05$; **significant at $P<0.01$; ns, not significant.

barley cultivars; and iii) both barley cultivars responded distinctly to inoculation with AM fungi. However, the commercial inoculum of *G. mosseae* had a better response with cultivar Julgeh and the commercial inoculum *R. irregularis* had a better response with cultivar Yusuf.

The main differences between two barley cultivars

Our findings illustrated a significantly higher plant yield and yield components following application of Zn in both investigated barley cultivars, but the increases were greater for cultivar Julgeh. This enhancement in Julgeh yield was expected with based on the responses in physiological and biochemical parameters including the increment in chlorophyll content and carbonic anhydrase activity in comparison with those of Yusuf under the same treatment. Indeed, cultivar Yusuf, displayed a higher potential and invested more in the development of root traits. Zn-deficient barley varieties have previously been shown to have greater root mass compared to shoot mass (Tiong *et al.,* 2015). As in our experiment, this has also been observed for wheat (Rengel and Romheld, 2000; Kumar, 2001). Our findings corresponded with these observations for Yusuf but to a lesser extent for Julgeh, which was the more efficient cultivar, based on higher shoot and yield parameters. Julgeh was more efficient than Yusuf based on the definition of Singh *et al*. (2005) because of its higher capacity to absorb Zn. We assume that the advantage in increasing the Zn concentration of Julgeh grain compared to that of Yusuf can also be traced to genetic variation between cultivars, in parallel with observations for wheat cultivars (Rengel and Romheld, 2000). Our findings for barley cultivars coincide with those of biochemical traits for wheat genotype with higher Zn efficiency, where the activity of carbonic anhydrase was two-fold greater than in Zn-inefficient genotypes (Rengel, 1995). Therefore, we hypothesize that the genetic dominance of Julgeh whether in its biochemical traits including the greatest chlorophyll content, carbonic anhydrase and soluble sugar or morphological parameters as shoot development may have led to the enhancement of its yield parameters.

The influence of nano and ordinary form of Zn foliar application on barley

Foliar application of Zn in crop production has been recommended as an effective way to compensate cereal yield and improve nutrient deficiency of this vital element, especially in severely deficient soils (Cakmak, 2009). As previously reported (Khan *et al*., 2014), interactions between P and Zn in the rhizosphere, especially due to the excessive use of P fertiliser, can result in an imbalance between the amounts of these essential elements in plant tissue, leading to Zn deficiency in shoots and grain. Therefore, it can be hypothesised that the reduction in crop productivity common in rain-fed and low fertility soils could be addressed with foliar application of nutrients, especially micronutrients, as suggested by Prasad *et al*. (2012). This was previously supported in a pot experiment (Genc *et al*., 2004) for two barley genotypes differing in Zn efficiency growing in deficient sandy soil. With application of different levels of Zn fertiliser rating from 0-12.8 (mg^{-1} kg dry soil), the concentration of Zn in grain

Table 4. Effects of Zn foliar application and inoculation with arbuscular mycorrhizal fungi on root and shoot parameters of two barley cultivars.

Cultivar	Mycorrhiza	Zn treatment	TRL (mm)	RA (mm^2)	ARD (mm)	RV (mm^3)	RD (cm)	RDW (g)	R/S	LA (cm^2)	LA/RA	SDW (g)	LDW (g)
Yusuf	Control	Zn1	2593^{l}	215.3^{jk}	0.61^{k}	7.2^{q}	57.24^{o}	0.74^{lmn}	0.74^{a}	258^{n}	1.20^{kl}	1.00^{q}	0.47^{m}
		Zn2	2946^{i}	231.3^{h}	0.92^{hi}	9.8^{k}	62.66^{j}	0.86^{jk}	0.57^{de}	295^{l}	1.28^{gh}	1.51^{m}	0.76^{g}
		Zn3	2673^{k}	224.0^{i}	0.64^{k}	8.5^{n}	58.81^{n}	0.78^{klm}	0.64^{bc}	260^{n}	1.16^{l}	1.22^{o}	0.53^{k}
		Zn4	2806^{j}	228.0^{h}	0.77^{j}	9.2^{mn}	60.59^{l}	0.83^{jkl}	0.59^{cd}	282^{m}	1.24^{ij}	1.40^{n}	0.63^{i}
	G. mosseae	Zn1	3133^{h}	238.6^{g}	0.66^{k}	7.7^{p}	71.96^{h}	1.00^{gh}	0.66^{b}	295^{l}	1.24^{ij}	1.76^{j}	0.63^{i}
		Zn2	3740^{e}	259.3^{d}	1.01^{f}	8.7^{m}	80.58^{e}	1.14^{cde}	0.46^{jk}	338^{hi}	1.31^{g}	2.48^{c}	0.89^{e}
		Zn3	3306^{g}	246.0^{f}	0.80^{j}	8.2^{o}	74.67^{g}	1.01^{gh}	0.50^{hi}	305^{k}	1.24^{ij}	2.02^{fg}	0.77^{g}
		Zn4	3526^{f}	252.0^{e}	0.91^{hi}	9.0^{mn}	78.59^{f}	1.02^{fgh}	0.45^{jk}	316^{j}	1.25^{hi}	2.25^{d}	0.82^{f}
	R. irregularis	Zn1	3906^{d}	266.6^{c}	0.81^{j}	8.3^{o}	84.17^{d}	1.11^{def}	0.56^{ef}	314^{j}	1.18^{kl}	1.98^{gh}	0.43^{n}
		Zn2	5060^{a}	288.0^{a}	1.02^{f}	9.7^{k}	92.46^{a}	1.31^{a}	0.45^{jk}	368^{ab}	1.28^{gh}	2.90^{a}	0.89^{e}
		Zn3	4160^{c}	276.0^{b}	0.89^{i}	8.9^{mn}	85.48^{c}	1.19^{bcd}	0.52^{gh}	336^{hi}	1.22^{jk}	2.28^{d}	0.83^{f}
		Zn4	4453^{b}	279.3^{b}	0.95^{gh}	9.1^{ln}	88.05^{b}	1.26^{ab}	0.47^{jk}	347^{ef}	1.24^{ij}	2.69^{b}	0.87^{d}
Julgeh	Control	Zn1	1793^{u}	166.6^{q}	0.76^{j}	9.5^{m}	48.14^{t}	0.63^{o}	0.57^{de}	319^{j}	1.71^{de}	0.95^{q}	0.31^{o}
		Zn2	2026^{s}	182.6^{o}	1.07^{e}	13.1^{i}	53.77^{q}	0.69^{no}	0.44^{kl}	347^{de}	1.62^{f}	1.55^{lm}	0.62^{ij}
		Zn3	1886^{t}	174.0^{p}	0.87^{i}	12.0^{kl}	49.51^{s}	0.64^{o}	0.56^{de}	331^{i}	1.75^{d}	1.14^{p}	0.42^{n}
		Zn4	1953^{t}	180.6^{o}	0.99^{fg}	12.2^{jk}	51.54^{r}	0.65^{o}	0.48^{ij}	345^{fg}	1.69^{e}	1.34^{n}	0.51^{l}
	G. mosseae	Zn1	2320^{op}	202.0^{l}	1.21^{d}	27.3^{c}	56.89^{o}	0.89^{ij}	0.50^{hi}	355^{de}	1.92^{b}	1.76^{j}	0.74^{h}
		Zn2	2493^{m}	223.3^{i}	1.53^{a}	29.6^{a}	66.00^{i}	1.23^{abc}	0.54^{fg}	377^{a}	1.98^{a}	2.27^{d}	1.15^{a}
		Zn3	2380^{no}	212.6^{k}	1.32^{c}	26.8^{d}	59.79^{m}	0.95^{hi}	0.49^{ig}	372^{ab}	1.90^{b}	1.94^{h}	0.90^{d}
		Zn4	2440^{nm}	217.3^{j}	1.44^{b}	28.6^{b}	63.32^{j}	1.08^{efg}	0.50^{hi}	366^{bc}	1.91^{b}	2.16^{e}	0.94^{b}
	R. irregularis	Zn1	2080^{rs}	183.3^{o}	0.89^{i}	21.4^{h}	55.41^{p}	0.72^{mno}	0.45^{jk}	336^{hi}	1.83^{c}	1.59^{l}	$0. 83^{f}$
		Zn2	2253^{pq}	195.3^{m}	1.23^{d}	24.3^{e}	61.88^{k}	0.81^{jkl}	0.39^{l}	362^{d}	1.93^{b}	2.06^{f}	0.73^{h}
		Zn3	2126^{r}	188.0^{n}	0.92^{hi}	22.5^{g}	57.28^{o}	0.75^{lmn}	0.45^{jk}	343^{gh}	1.82^{c}	1.66^{k}	0.52^{kl}
		Zn4	2200^{q}	192.0^{m}	1.03^{ef}	23.7^{f}	59.59^{m}	0.77^{lmn}	0.42^{kl}	355^{de}	1.85^{c}	1.84^{i}	0.61^{j}
		LSD (0.05)	71.349	3.3954	0.054	0.0404	0.7302	0.0885	0.0544	8.5128	0.0501	0.0584	0.019

In the same column, values marked with the same letters are similar ($P<0.05$), whereas those with different letters are significantly different. Zn1= no spraying; Zn2 = nano ZnO; Zn3= ordinary ZnO; Zn4 = nano + ordinary ZnO. TRL, total root length; RA, root area; ARD, average root diameter; RV, root volume; RD, root depth; RDW, root dry weight; R:S, root:shoot ratio; LA, leaf area; LA:RA, ratios of leaf area to root area; SDW, shoot dry weight; LDW, leaf dry weight; LSD, least significant difference.

and the grain yield in the more efficient genotype were significantly increased compared to those of the Zn-inefficient genotype. They stated that the improvement in the Zn efficient cultivar, which is occurred in all level of Zn fertilisers even under Zn deficiency, was due to high translocation of Zn from vegetative plant parts to grain. Similarly, our observation that foliar application of Zn to barley leaves improved grain Zn concentration, grain yield, thousand kernel weight and harvest index parameters for Julgeh more than for Yusuf. These differences may be due to genotypic variation between cultivars in their Zn absorption capability due to differences in leaf structure in favour of Julgeh. These findings were in accordance with a study by Painkra *et al.* (2015) where the concentration of Zn in rice grain was significantly influenced by genotypes and Zn. Eleiwa *et al*. (2013) also reported that foliar application of Zn influenced barley vegetative growth including plant height, spike length, tillering, leaf number and area, as well as photosynthesis pigments (chlorophyll a, b and carotenoids). Thus, an appropriate concentration of ZnO has potential to improve growth of barley by promoting physiological parameters as chlorophyll, photosynthesis and essential growth enzymes. Foliar application of Zn would therefore be a plausible strategy to overcome the lack of Zn in plants as recently reported by Yagmur *et al*. (2017) where there was remobilization and loading of Zn from leaves to grain of barley cultivars following spraying with Zn under field conditions. We demonstrated that among the forms of Zn applied as a foliar spray to the two barley cultivars in our experiment, the nano form of Zn lead to the highest concentrations of chlorophyll and soluble sugar in leaves. This is in line with a previous exploratory study reporting that nanoparticles below100 nm possess high potential to penetrate plant cells through either stomata or the vascular system (Eichert *et al.*, 2008) due to their smaller particle size, in both diameter and weight (Fedorenko *et al*., 2015), greater solubility in water and more efficiency compared to ordinary forms of Zn (Joseph and Morrison, 2006). Previous studies also showed that foliar application of ZnO nanoparticles increased Zn concentration in grain of maize (Subbaiah *et al*., 2016) and durum wheat (Deshpande *et al*., 2018). In our study, the nano form of Zn also lead to a higher concentration of Zn in grain and to greater carbonic anhydrase activity in leaves and grain phytase activity for cultivar Julgeh. A significant increase in the root and shoot morphological traits of both barley cultivars with application of the nano compared to ordinary form of Zn is another characteristic of this novel Zn fertiliser. Application of ZnO in the form of nanoparticles (20 ppm) to *Cicer ariatium* (chickpea) seedlings enhanced root and shoot biomass by about 42% and 98% respectively (Mahajan *et al*., 2011). For Pearl Millet, root area increased with foliar application of nano Zn fertiliser (Tarafdar *et al*., 2014). But contradictory results were associated with the yield and yield related parameters of both cultivars indicated that the foliar spraying of Zn with significant increase in yield and yield component occurred with the advantage of ordinary form compared to the nano. Somewhat different results were obtained by Janmohammadi *et al*. (2016) for barley in which they found that the foliar application of nano fertiliser in a combination of Zn nano-chelate with 2000 ppm nano-TiO_2 significantly influenced barley traits and yield components compared with the ordinary form when grown in the field. Tarafdar *et al.* (2014) also reported significant improvement in grain yield and Zn concentration for Pearl Millet following foliar application of nano Zn. While foliar application of the nano form of Zn influenced root and shoot growth, and all biochemical traits of barley in our study, it is difficult to understand the basis of a preferential response of yield improvement compared with the ordinary form of Zn foliar application. One possibility is that since for improvement of yield and yield components in crop there is a need for use of optimum concentration of nano fertilisers Singh *et al*. (2017), the application of nano Zn less than optimum may enable to achieve priority to increase traits related to yield in comparison to ordinary form. Therefore, we hypothesized that optimum applied dose and concentration of ZnO more than applied doses in this study could enhance the yield parameters as well as morpho-physiological traits. Further research to determine the appropriate dose of foliar application of nano Zn on the barley plant is required. Nevertheless, it reflects a high potential for optimum doses of Zn nano fertiliser, which is likely to be environmentally friendly and affordable (Tarafdar *et al*., 2014) with potential to improve crop production and increase essential micronutrients in grain.

The influence of mycorrhizal inoculation on barley

In our study, inoculation with commercially available AM fungi, especially *G. mosseae* with Julgeh and *R. irregularis* with Yusuf, positively enhanced the Zn concentration in barley grain by 192% and 159% respectively in plants grown in pots under field conditions. Improvements in grain quality with strategies to enhance the concentration of Zn in grain lead to better root and shoot traits compared to those arising from low Zn content in seeds (Cakmak, 2008). This will ensure improvement in seed vigour and seed germination in the next generation (Kinaci and Kinaci, 2005). Furthermore, seedlings that emerge from seeds with a high level of Zn can reinforce their tolerance and resistance in adverse environmental circumstances (Mousavi, 2011). According to the findings described by Kothari *et al*. (1991), maize plants inoculated with an AM fungus increased Zn concentration in shoots by 164%. Similarly, for wheat, mycorrhizal colonization increased with increases in root, shoot and grain Zn (Zhang *et al*., 2016). The enhancement in the concentration of Zn and P in wheat grain, grain yield and the strong positive correlation between inoculation with AM fungi and the aforementioned traits was revealed from the meta-analysis conducted by Pellegrino *et al*. (2015).

Improvement in grain quality following inoculation with AM fungi could be due to enhanced nutrient availability or to enhancement of the microbial population in the rhizosphere (Barea *et al*., 2005). In our study, Yusuf inoculated with *R. irregularis* when it was sprayed with nano ZnO showed greater potential to increase total root length, root area, root depth, root dry weight than did cultivar Julgeh which is more likely to invest more of its assimilate on shoot parameters instead of greater belowground investment. Similiarly, Subramanian *et al.* (2009) illustrated significant increases in morphological root traits including root length (vertical), root spread (horizontal), root volume and physiological parameters including chlorophyll content and grain Zn concentration in inoculated maize plants compared with un-inoculated plants and highlighted that this improvement was re-enforced with the application of Zn in the form of $ZnSO_4$ fertilisation to the soil. Apart from the advantages of the mycorrhizal symbiosis, the increment in root traits with application of Zn may due to the vital role played by Zn in auxin synthesis. In recent observations of rice seedlings under Zn deficiency, auxin production decreased resulting in a significant reduction in morpho-physiological traits and also root growth (Begum *et al*., 2016). Our findings suggest that cultivar Yusuf with greater belowground investment particularly in vertical development of root traits such as total root length and root depth may be better able to take advantage for water and nutrient acquire from the deeper layers of the soil. Therefore, we hypothesis that cultivar Yusuf with its vertical root development and deeper root structure is better suited as a dryland cultivar in arid to semi-arid environments. Similarly, Koevoets *et al*. (2016) showed that crops with thinner and deeper root systems or higher root: shoot ratio are more likely to acquire more water from deeper soils, which could be an adaptation to alleviate the impact of drought during production of those crops.

We found that inoculation with *G. mosseae* highlighted a high capacity to increase horizontal development of the root system of cultivar Julgeh with improvements in root diameter and root volume. Feddermann *et al*. (2010) also observed that plants inoculated with AM fungi had reduced root: shoot biomass ratio due to allocation of more biomass to shoots. They concluded that this allometric relationship between roots and shoots increased carbon assimilation to meet the demand of the AM fungi (Feddermann *et al*., 2010). According to Nouri *et al*. (2014) there is a reciprocal relationship between the amount of elements such as N and P and fungal coexistence, so that deficiency in these two macro elements strengthens the symbiotic relationship and increases colonisation of the host plant. A low rate of root colonisation in non-mycorrhizal plants can be due to increased P (Liu *et al.*, 2016). However, foliar application of Zn could address Zn deficiency caused by interactions between Zn and P in soil (Mousavi *et al*., 2011), and AM fungi could increase the efficiency of use of P fertiliser. Thus, co-inoculation with AM fungi and ordinary foliar application of ZnO could be an agronomic practice for effective barley production and the nano form of Zn could contribute to higher Zn concentration in barley grain. The percentage of mycorrhizal colonisation has not been measured in this study.

Conclusions

The two cultivars of barley investigated here differed in response to combinations of the form of Zn applied as a foliar spray and inoculation with two commercial inocula of AM fungi. Foliar Zn application in the nano form significantly enhanced root and shoot morphological and physiological traits, and increased the concentration of Zn in barley grain. The barley cultivar Julgeh inoculated with *G. mosseae* had physiological traits that were more likely to enhance yield and yield parameters than those of barley cultivar Yusuf that invested more in root traits and vegetative growth. The extent of benefit of mycorrhizal inoculation for each barley cultivar depended on the form of foliar Zn applied. The unique approach of nano Zn in the form of foliar fertiliser could elevate low concentration of Zn in barley grain, ensure the quality and vigor of seedlings in Zn-deficient soils, and eliminate the need for application of fertiliser Zn to the soil. Therefore, application of nano foliar fertiliser and exploitation of naturally occurring AM fungi have potential to contribute to sustainable agricultural production by reducing the requirement for application of synthetic chemical fertilisers. Hence, a plausible strategy would be to select appropriate levels and forms of P fertiliser based on soil and crop requirements. This would contribute to P management, nutrient use efficiency, and improved contributions from the microbial community in soil. Such a fertiliser management approach, along with the Zn spraying, could make a positive step towards increasing the content of Zn in grain and its quality. Overall, foliar Zn application and mycorrhizal inoculation could contribute to the quality and quantity of barley grains, especially under arid and semi-arid regions.

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