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How important is the mycorrhizal pathway for plant Zn uptake?

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How important is the mycorrhizal pathway for plant Zn uptake?

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Abstract

Introduction Formation of arbuscular mycorrhizas can enhance plant uptake of immobile nutrients such as zinc and phosphorus. Enhancement of Zn uptake by arbuscular mycorrhizal (AM) fungi on Zn-deficient soils has been studied previously, however, the quantity of Zn that is contributed by the AM pathway of uptake to the plant has not previously been reported for soil of any Zn status.

Methods We grew a mycorrhiza-defective mutant tomato (*Solanum lycopersicum* L.) genotype (*rmc*) and its mycorrhizal wild-type progenitor (76R) in pots containing a hyphal compartment (HC) accessible only by the external hyphae of AM fungi, and containing the radioisotope ^{65}Zn. This was repeated at three soil Zn concentrations, ranging from low to high. We estimated the amount of Zn delivered *via* both the AM and direct (root) pathways.

Results Up to 24% of Zn in the shoots of the AM plants was delivered *via* the AM pathway at the lowest soil Zn treatment. This decreased significantly, to 8%, as soil Zn concentration increased. No ^{65}Zn was detected in the tissues of the non-mycorrhizal genotype.

Conclusions The relative contribution to shoot Zn by the AM pathway of uptake was highest when soil Zn was low, and decreased with increasing soil Zn concentration.

Introduction

It is estimated that 50% of the world's important cereal growing soils are considered low in plant-available Zn (Cakmak 2002; Graham and Welch 1997) and, thus, plant Zn deficiency is common and widespread (Hacisalihoglu and Kochian 2003). In turn, this can have important implications for human nutrition; indeed 30% of the world's population is affected by Zn deficiency (Alloway 2008). On the other hand, Zn can also be present in soils at levels toxic to plants (Jung and Thornton 1996). Therefore, there is need for a fundamental understanding of the factors that regulate plant Zn acquisition.

Most terrestrial plants form arbuscular mycorrhizas, and these associations between plant roots and a specialised group of soil fungi enhance the capacity of plants to acquire nutrients (Smith and Read 2008). The role of mycorrhizas in uptake of soil Zn is dependent on the concentration of Zn in the soil (Chen et al. 2003; Watts-Williams et al. 2013). It is well established that under low Zn conditions, plants that form mycorrhizas often have higher Zn concentrations and contents compared to non-mycorrhizal plants (Cavagnaro 2008). Interestingly, it has also been reported that where soil Zn concentrations are toxic to plants, the formation of mycorrhizas can 'protect' plants against excess Zn uptake compared to non-mycorrhizal plants growing in the same soil (Chen et al. 2003; Christie et al. 2004; Watts-Williams et al. 2013). Thus, mycorrhizas have an important role to play in modulating plant Zn acquisition under a wide range of soil Zn concentrations.

There are two pathways of soil-derived uptake for most mineral nutrients (including Zn and P), in AM plants: directly *via* the root epidermis (direct pathway of uptake;

DPU), and *via* the mycorrhizal pathway of uptake (MPU). Radioisotopes of Zn (e.g. ^{65}Zn) have been used in previous studies to trace AM uptake (discussed below), but none have quantified the proportion or amount of Zn taken up by the MPU. Kothari et al. (1991) presented an estimate of 16-25% for the minimum contribution of Zn by the external hyphae of AM fungi, although they did not use radioisotope tracing or dilution. Additionally, a number of studies have demonstrated the ability of the external hyphae to translocate ^{65}Zn from soil and agar media, but presented results in units that quantify the relative activity (e.g. in counts per minute) of ^{65}Zn in different treatments, rather than the proportion of a plant's Zn delivered *via* the MPU (Bürkert and Robson 1994; Mehravaran et al. 2000). Jansa et al. (2003) quantified MPU Zn contribution by the proportion of added ^{65}Zn that was transported to the plants, and found that it was much higher than the estimates from other studies. However, the quantity of Zn that is delivered *via* the MPU and DPU (i.e., in µg of Zn), remains to be reported.

There are numerous studies examining how much P is delivered through the MPU, compared to the DPU, using radioisotopes of phosphorus (P) (Grønlund et al. 2013; Jakobsen et al. 1992; Joner and Jakobsen 1994; Pearson and Jakobsen 1993; Poulsen et al. 2005; Schweiger and Jakobsen 1999; Smith et al. 2004; Thingstrup et al. 2000). It has been demonstrated that between 20 and 100% of a plant's P can be delivered via the MPU, depending on plant and AM fungi species (Smith et al. 2004). While the relative importance of the two pathways (i.e., proportion of nutrients entering *via* the pathways) has been well established for P, it has not been elucidated for other nutrients, including Zn; however, it is of high priority (Cavagnaro 2014). That is, we

do not know how much Zn enters the plant *via* the AM pathway, be it at low, adequate, or toxic soil Zn concentrations.

On the basis of these previous results, we designed this study with two specific aims:

i) To quantify the amount of Zn taken up *via* the MPU using a radioisotope of Zn; and

ii) To investigate whether the contribution to Zn uptake *via* the MPU changes with soil Zn concentration.

We hypothesised that a significant proportion of plant Zn will be delivered *via* the MPU at low concentrations of Zn in soil, but that this may decrease as soil Zn concentration is increased, and direct uptake increases.

Materials and Methods

Soil and plant preparation

Plastic pots were filled with 1.4 kg of a 90:10 (w/w) sand/soil mixture that included 140 g of *Rhizophagus irregularis* AM fungal inoculum. The soil/sand mix was comprised of washed sand and soil collected from the Mallala region of South Australia, used in prior studies (see Cavagnaro et al. 2001 for details). The soil/sand mixture was autoclaved and sieved to <2 mm prior to use. The soil was amended with one of three $ZnSO_4.7H_2O$ additions, at the rates of 2, 20, and 50 mg Zn kg $soil^{-1}$, referred to as "Low Zn", "Medium Zn" and "High Zn", hereafter (Table 1). Supplemental P was also added to the soil in all treatments at a rate of 25 mg anhydrous $CaHPO_4$ kg $soil^{-1}$, in order to stimulate plant growth without inhibiting AM colonisation (Cavagnaro et al. 2010). Following supplemental P addition, total P

concentration (see below for details) of the soil was 27.8 ± 1.2 mg P kg^{-1} soil, and resin-extractable P concentration was 2.0 ± 0.09 mg P kg^{-1} soil.

To quantify the contribution of the MPU to shoot Zn, hyphal compartments (HCs) containing radioisotope labelled soil were added to the pots, modified from Jakobsen et al. (1992) (see Fig. 1) as follows. The HCs were capped at one end with nylon mesh having a 25 μm pore diameter and filled with 40 g of soil which had been labelled with 684 ± 18 kBq of ^{65}Zn (Perkin-Elmer, U.S.A.), followed by 10 g of unlabelled soil. One HC was placed in each pot of corresponding soil Zn addition treatment, with the nylon mesh side facing inwards. Soil from "extra" HCs, that were kept in pots containing moist soil for the duration of the experiment without plants growing, were analysed for Zn concentration and ^{65}Zn activity. Total Zn concentration was measured on oven-dried (105 °C for 48 hours) soil samples that were digested with *aqua regia*, according to Zarcinas et al. (1996). Dried soil from "extra" HCs was also subsampled for determination of plant-available (DTPA-extractable) Zn (Lindsay and Norvell 1978) and plant-available (resin-extractable) P (McLaughlin et al. 1994). Measurement of Zn and P concentrations, and ^{65}Zn activity, in the digests and extracts was performed by inductively-coupled plasma atomic emission spectrophotometry (ICP-AES, Spectroflame Modula, Spectro, Germany) and γ-spectroscopy (1480 Wizard TM3®, Wallac, Germany) respectively. These data were used in the calculation of Zn uptake *via* the MPU (see below).

Seeds of the reduced mycorrhiza colonisation tomato (*Solanum lycopersicum* L.) mutant genotype (*rmc*, hereafter) and its mycorrhizal progenitor (76R, hereafter) were surface-sterilised and pre-germinated on moist filter paper for 5 days (following

Cavagnaro et al. 2010). The *rmc* genotype has been previously established as an appropriate non-mycorrhizal control when compared to its wild-type 76R (Barker et al. 1998; Watts-Williams and Cavagnaro 2014). Four pre-germinated seeds were planted into each pot and, after one week, were thinned to one seedling per pot. Treatments were replicated five times. Plants were grown in a controlled environment glasshouse at The University of Adelaide, Waite campus, during February-April, 2014. Over this period, mean minimum temperature in the glasshouse was 19.3 ± 0.27°C, and mean maximum temperature was 24.9 ± 0.27°C. Mean light level during the day was 369 μmol photons m^{-2} s^{-1}. Plants were watered twice weekly with deionised (DI) water, and once weekly with 1/10 strength modified Long Ashton solution (P and Zn omitted, following Watts-Williams et al. 2014) to 10% soil weight. The pots were arranged on the glasshouse bench in a randomised complete block design and were re-randomised at each watering event. Four weeks after planting, each plant received 10 mg P and 10 mg N, following the appearance of P-deficiency and N-deficiency symptoms in the shoots.

Harvesting and sample analysis

All plants were destructively harvested 53 days after planting, as follows. Shoots were separated from roots and weighed. Roots were washed free of loose soil, weighed, and subsampled for determination of AM colonisation. All remaining biomass was oven dried at 55 °C before being weighed. Dried shoot and root biomass was subsampled and then digested with concentrated nitric acid, according to Zarcinas et al. (1987). Plant digests were analysed for total P and Zn concentrations by ICP-AES, Spectroflame Modula, Spectro, Germany) and for ^{65}Zn activity by γ-spectroscopy (1480 Wizard TM3®, Wallac, Germany).

160

161 *Calculations and data analysis*

162 Specific activities in soil and plant tissue were calculated using the following
163 equations (as established for P, modified from Smith et al. 2004):

164 Eqn 1a:

$$\text{Shoot specific activity} = \frac{^{65}\text{Zn activity (kBq) g}^{-1}\text{ shoot dry weight}}{\text{Zn (µg) g}^{-1}\text{ shoot dry weight}}$$

165

166 Eqn 1b:

$$\text{Soil specific activity} = \frac{^{65}\text{Zn activity (kBq) g}^{-1}\text{ dry soil}}{\text{Zn (µg) g}^{-1}\text{ dry soil}}$$

167

168 We calculated mycorrhiza-mediated contribution to shoot Zn (% and µg Zn) using the
169 SA values determined using the DTPA-extraction solution. However, as the DTPA-
170 extraction method overestimates plant-available zinc, we have presented contribution
171 to shoot Zn by mycorrhizas as a range between the values calculated from the DTPA
172 data and the 'corrected' DTPA (for explanation, see Discussion).

173

174 The relative proportional AM contribution to shoot Zn uptake (%) was calculated as:

175 Eqn 2:

$$\text{Percent contribution to shoot Zn (\%)} = \frac{\text{Eqn 1a}}{\text{Eqn 1b}} \times \frac{\text{Total soil weight}}{^{65}\text{Zn labelled soil weight}} \times 100$$

176

177 Mycorrhizal contribution to shoot Zn uptake (mg Zn) was calculated as:

178 Eqn 3:

$$\text{Mycorrhizal contribution to shoot Zn (µg Zn)} = \frac{\text{Shoot Zn content (µg)} \times \text{Eqn (2)}}{100}$$

In the interests of not over-estimating plant Zn uptake *via* the MPU, we calculated MPU contribution to shoots and not roots. This was because the activity of ^{65}Zn and non-radioactive Zn originating from the HC may be bound in external fungal structures that cannot be separated from ^{65}Zn activity or Zn content in the dry root biomass. Thus, shoot ^{65}Zn activity and Zn content data are a more reliable indicator of Zn uptake *via* the MPU.

All response variables were analysed by two-way ANOVA with *Genotype* and *Zn addition treatment* as factors in the analyses, with the exception of AM colonisation of roots and AM contribution to shoot Zn, which were analysed by one-way ANOVA in the 76R genotype only, with *Zn addition treatment* as the factor. Where significant differences were found, comparisons were made using Tukey's honestly significant difference (HSD). We did not include *rmc* data in the statistical analysis of AM colonisation or AM contribution to shoot Zn because *rmc* roots were not colonised and had no ^{65}Zn activity (discussed below). All statistical analyses were performed using JMP (Version 10.0.0, SAS Institute Inc., Cary, NC).

Results

Mycorrhizal colonisation

Roots of the *rmc* genotype were not colonised by AM fungi. In contrast, the roots of the 76R genotype were well colonised by AM fungi (Table 2). Mean AM colonisation in the 76R genotype was 37.8 ± 2.4% root length colonised across all Zn addition

treatments, and there were no significant differences among the Zn addition treatments (Table 3).

Plant biomass

For shoot dry weight (SDW; Table 2), and total dry weight (TDW), there was a significant main effect of *Genotype*, with *rmc* plants having a significantly higher SDW and TDW than 76R plants (TDW was 2.24 and 2.04 g, respectively), irrespective of *Zn addition treatment* (Table 3). For root dry weight (RDW) there was a significant main effect of *Zn addition treatment*, with the RDW significantly higher at High Zn than at both Medium Zn, and Low Zn, irrespective of *Genotype*.

Plant Zn and P nutrition

The interaction between *Genotype* and *Zn addition treatment* was not significant for shoot or root Zn content (Table 3). However, both shoot and root Zn contents increased with increasing soil Zn addition, with the differences being significant between Low and Medium Zn, and between Medium and High Zn, irrespective of *Genotype* (Fig. 2a,b). The same pattern was observed in shoot and root Zn concentration data (Table 2), except that there was a significant main effect of *Genotype* on root Zn concentration (Table 3), whereby the AM genotype had significantly higher root Zn concentration than the non-mycorrhizal genotype, pooling *Zn addition treatment*.

Shoot and root P contents did not change in response to soil Zn addition (Fig. 3a,b), and there was no significant interaction between *Genotype* and *Zn addition treatment*. However, in the roots only, there was a significant main effect of *Genotype*, whereby

227 the 76R genotype had significantly higher root P content than the *rmc* genotype,
228 irrespective of *Zn addition treatment*.
229
230 *Mycorrhizal contribution to plant Zn (% and µg Zn)*
231 The activity of ^{65}Zn in the *rmc* plants was minimal and was not significantly greater
232 ($P>0.05$, student's *t*-test) than background activity (data not shown), confirming that
233 there was no 'leakage' of ^{65}Zn out of the HC. By contrast, the activity in the 76R
234 plants was significantly ($P<0.05$, student's *t*-test) one to two orders of magnitude
235 greater than background activity. This indicates that the HC method was effective in
236 excluding roots, and that external hyphae of AM fungi were able to colonise the HC
237 and acquire and deliver Zn to the plants. For this reason, and the absence of AM
238 colonisation in the *rmc* genotype, we excluded the data for *rmc* from the following
239 analyses.
240
241 Up to 24.2% of the Zn entering the shoots of the 76R genotype was delivered *via* the
242 MPU in the Low Zn treatment (Fig. 4a). Further, mycorrhizal contribution to shoot Zn
243 was relatively constant, while direct pathway Zn uptake increased dramatically as soil
244 Zn addition increased. Taken together, the relative contribution *via* the MPU
245 decreased significantly with increasing soil Zn concentration. Specifically, the relative
246 contribution by mycorrhizas to shoot Zn (%) in the 76R genotype was significantly
247 lower at High Zn than at Low or Medium Zn. The greatest contribution by
248 mycorrhizas to shoot Zn was 21.7 µg, in the Medium Zn treatment, however the MPU
249 contribution to shoot Zn (in µg Zn) was not significantly different among Zn addition
250 treatments (Fig. 4a). Values of DPU in the shoots of AM plants increased with
251 increasing Zn concentration in terms of both proportion and amount of Zn (Fig. 4a-b).

Discussion

The aims of this study were to: 1) quantify the contribution by AM fungi to shoot Zn uptake in the 76R tomato genotype, and 2) investigate whether contribution by AM fungi to total shoot Zn uptake changes with increasing soil Zn concentration. We found that at low soil Zn, the relative contribution by the AM fungus to shoot Zn was up to 24%, and this decreased significantly with increasing soil Zn concentration, as uptake *via* the direct uptake pathway increased.

Mycorrhizal colonisation and plant biomass

As expected, the roots of the *rmc* plants were not colonised by AM fungi, and levels of colonisation in the 76R genotype were comparable with previous studies in the same, and a different, soil (Watts-Williams and Cavagnaro 2012; Watts-Williams et al. 2013). However, there was no effect of Zn fertilisation on mycorrhizal colonisation in this study, which has been previously demonstrated in a study that used the same soil (Cavagnaro et al. 2010).

As in previous studies using these genotypes, there was a small growth depression in the AM plants (Cavagnaro et al. 2008; Watts-Williams et al. 2013). This could be attributed to a carbon drain on the AM plants as a result of the fungal colonisation (Johnson et al. 1997), but this result is not important with respect to the calculations for the MPU for Zn in the present experiment.

Contribution by AM fungal uptake at low Zn

The DTPA-extraction process can over-estimate the plant-available fraction of soil Zn as it extracts not only part of the plant-available pool but also significant amounts of

278 the Zn pools unavailable to plants (Sinaj et al. 2004). According to Sinaj et al. (2004),
279 DTPA extracts twice the amount of soil Zn than is actually plant-available, across a
280 wide range of soils. However, Tiller et al. (1972) showed that soil Zn extraction with
281 EDTA (a chelating agent similar to DTPA) is able to equilibrate with the same form
282 of Zn taken up by plants from soils. Thus, we have presented contribution to shoot Zn
283 via the MPU as a range between the values calculated from the DTPA data and the
284 DTPA values 'corrected' according to Sinaj et al. (2004).
285
286 We found that a substantial proportion of shoot Zn entered *via* the MPU. The size of
287 the contribution of the MPU to plant Zn uptake has not previously been quantified. In
288 this study, we found that the maximum contribution to shoot Zn *via* the MPU was
289 24%, in the lowest soil Zn addition treatment. However, colonisation by AM fungi did
290 not significantly increase uptake of Zn by plants (Zn content) relative to the non-
291 mycorrhizal genotype at Low Zn, as has been found in other studies (Watts-Williams
292 et al. 2013). These results suggest that the activity of the mycorrhizal pathway of Zn
293 uptake can be masked by tissue Zn content values in AM- and non-mycorrhizal plants
294 unless the MPU and DPU are estimated separately, as in this study. That is, while the
295 MPU appeared inactive when we simply compared shoot Zn content of the
296 mycorrhizal and non-mycorrhizal genotypes, it actually delivered up to 24% of the
297 mycorrhizal plant's shoot Zn.
298
299 Values of MPU contribution are no doubt influenced by many factors, including:
300 plant species, soil type and chemistry, AM fungal isolate and inoculum potential, size
301 of the HC, days grown, and soil nutrient (particularly Zn and P) availability.
302 Furthermore, the calculations of soil specific activity are highly dependent on the

303 method of determination of soil Zn concentration and ^{65}Zn availability. However, the
304 values presented here serve as a point of reference for future experiments of this
305 nature that use other plant, soil and AM fungus combinations.
306
307 In this study there was a positive mycorrhizal P response, independent of soil Zn
308 concentration (as indicated by Fig. 3). This is in line with other studies using these
309 genotypes using this (Cavagnaro et al. 2010), and other (Watts-Williams et al. 2014),
310 soils. Beyond this, we cannot speculate on the activity of the DPU and MPU for P
311 here, as we did not separate the two pathways. Jansa et al. (2003) separated the DPU
312 and MPU for P and Zn simultaneously and found that a much higher proportion of the
313 added ^{33}P was transported to the plant than the added ^{65}Zn (12.1% and 4.3%,
314 respectively), which supports our hypothesis. However, we have built on this work by
315 quantifying the relative contribution and amount of Zn taken up *via* the DPU and
316 MPU. In addition, interactions between P and Zn uptake by AM fungi have been
317 demonstrated in other studies without separating the pathways of uptake, using these
318 genotypes (Watts-Williams and Cavagnaro 2014; Watts-Williams et al. 2013) and
319 other plant species (Lambert et al. 1979; Liu et al. 2000). Thus, we conclude that the
320 interplay between the DPU and MPU of Zn and P is highly complex and would
321 benefit from further research where the uptake pathways for both nutrients are
322 separated.
323
324 *Contribution by AM over a range of soil Zn concentrations*
325 While shoot and root Zn uptake (i.e., content per plant) increased in accordance with
326 increasing soil Zn addition in both genotypes, the relative contribution of shoot Zn
327 (%) *via* the MPU in the AM genotype decreased at High Zn (Fig. 4). As the amounts

(μg Zn) taken up by the MPU did not decrease, the increased total uptake was due to the large increase in direct uptake by the roots. At low soil Zn supply, Zn is a diffusionally limited nutrient for plant uptake (Wilkinson et al. 1968), thus colonisation by AM fungi is most advantageous to plants when the soil is Zn-limiting (Cavagnaro 2008). However, in this study, as soil Zn supply increased, the diffusional limitation likely disappeared, allowing the roots to take up soil Zn freely *via* the direct pathway, thus explaining the observed increase in DPU activity. Previously, it has been suggested that AM colonisation can reduce plant Zn uptake when Zn is present in toxic levels, as illustrated by negative AM Zn responses (i.e., tissue Zn content less in AM plants than non-mycorrhizal plants) (Watts-Williams et al. 2013), reduced tissue Zn concentration (Cavagnaro et al. 2010; Li and Christie 2001; Lingua et al. 2008; Zhu et al. 2001), and reduced translocation of Zn to shoots and increased biomass (due to increased P uptake) (Chen et al. 2003) compared to the non-mycorrhizal state. In the present study, we did not observe reduced Zn content or increased biomass in the AM genotype at High Zn, but the relative proportion of Zn delivered by the MPU, and transferred to the shoots of the AM genotype, was significantly reduced at High Zn. The soil Zn additions chosen for this experiment were intended to represent 'low' and 'high' soil Zn concentrations, rather than 'deficient' and 'toxic' concentrations. However, future experiments in this area would benefit from using applications of soil Zn that are deficient and toxic to plants.

Conclusions and implications

We have shown that the MPU is considerably active in terms of Zn uptake at low soil Zn supply. This could have important implications for crops growing on Zn depleted soils. We also demonstrated that the relative contribution by the MPU decreased

353 substantially, as contribution by the DPU increased, as soil Zn supply increased.
354 Furthermore, comparison of plant Zn content between mycorrhizal and non-
355 mycorrhizal plants, or calculation of mycorrhizal Zn responses (MZnR), cannot tell us
356 about the activity of, and interplay between, the MPU and DPU. Separation of the two
357 pathways is required for this.
358
359 *Future directions*
360 Subsequent studies that utilise ^{65}Zn to trace mycorrhizal uptake of Zn in plants will be
361 useful, especially in conjunction with studies on P uptake (using ^{33}P or ^{32}P) *via* the
362 MPU and DPU. Furthermore, studies that focus on the expression of Zn transporter
363 genes will complement such physiological work. Specifically, identification of, and
364 investigation into, the expression of Zn transporter genes for both the MPU and DPU
365 in many plant species will be highly informative, as has been done for P. Stable- or
366 radio-isotopes have also been used to investigate mycorrhiza-mediated uptake of other
367 nutrients, including nitrogen (Johansen et al. 1992; 1993), sulphur (Rhodes and
368 Gerdemann 1978a; b) and calcium (Rhodes and Gerdemann 1978c); however, the
369 MPU contribution has not been quantified. Studies that quantify the MPU and DPU
370 uptake of these, and other, nutrients will be important in the ongoing study of
371 mycorrhizas and plant nutrition.
372
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Table 1. Zinc concentration, ^{65}Zn concentration and specific activity of soils, according to three methods of Zn quantification (see Calculations section in Methods for explanation of 'corrected' values)

		Low Zn	Medium Zn	High Zn
DTPA-extractable Zn	Zn conc. (μg g^{-1})	1.0	9.0	22.4
	^{65}Zn conc. (kBq g^{-1})	6.9	8.2	9.5
	Specific activity (kBq μg^{-1})	6.98	0.91	0.42
Corrected DTPA-extractable Zn	Zn conc. (μg g^{-1})	0.49	4.51	11.20
	^{65}Zn conc. (kBq g^{-1})	6.9	8.2	9.5
	Specific activity (kBq μg^{-1})	13.97	1.83	0.85
Total Zn	Zn conc. (μg g^{-1})	7.5	20.5	49.8
	^{65}Zn conc. (kBq g^{-1})	17.7	15.1	18.5
	Specific activity (kBq μg^{-1})	2.35	0.74	0.37

Table 2. Mycorrhizal colonisation of the mycorrhizal 76R tomato (*Solanum lycopersicum*) genotype, shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW), and shoot and root Zn concentrations (μg g^{-1}) of the 76R and *rmc* genotypes. *n*=5.

			Mycorrhizal colonisation (%)	SDW (g)	RDW (g)	TDW (g)	Shoot Zn conc. (μg g^{-1})	Root Zn conc. (μg g^{-1})
76R	Low Zn	mean	42.6	1.39	0.66	2.05	38.3	30.2
		s.e.	2.7	0.04	0.03	0.07	2.1	1.8
	Medium Zn	mean	30.4	1.30	0.71	2.01	74.3	94.4
		s.e.	2.6	0.04	0.05	0.05	2.2	3.7
	High Zn	mean	40.4	1.29	0.78	2.07	125.4	206.8
		s.e.	5.0	0.09	0.05	0.13	6.6	10.5
rmc	Low Zn	mean		1.46	0.79	2.25	31.8	20.9
		s.e.		0.07	0.03	0.08	1.4	1.6
	Medium Zn	mean		1.51	0.66	2.17	70.2	72.0
		s.e.		0.02	0.03	0.04	6.1	3.3
	High Zn	mean		1.49	0.82	2.31	119.2	186.7
		s.e.		0.02	0.04	0.05	2.4	10.2

Table 3. ANOVA summary table for all response variables.

	Zn	*G*	*Zn*G*
Mycorrhizal colonisation	ns	-	-
DTPA Zn AM contribution (%)	*	-	-
DTPA Zn AM contribution (μg Zn)	ns	-	-
Shoot dry weight (SDW)	ns	**	ns
Root dry weight (RDW)	*	ns	ns
Total dry weight (TDW)	ns	**	ns
Shoot P content	ns	ns	ns
Root P content	ns	**	ns
Shoot Zn content	***	ns	ns
Root Zn content	***	ns	ns
Shoot Zn concentration	***	ns	ns
Root Zn concentration	***	**	ns

Factors in the analysis were *Zn* (*Zn addition treatment*) and *G* (*Genotype*). Both the main effects and interaction term are indicated where relevant. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.0001$.

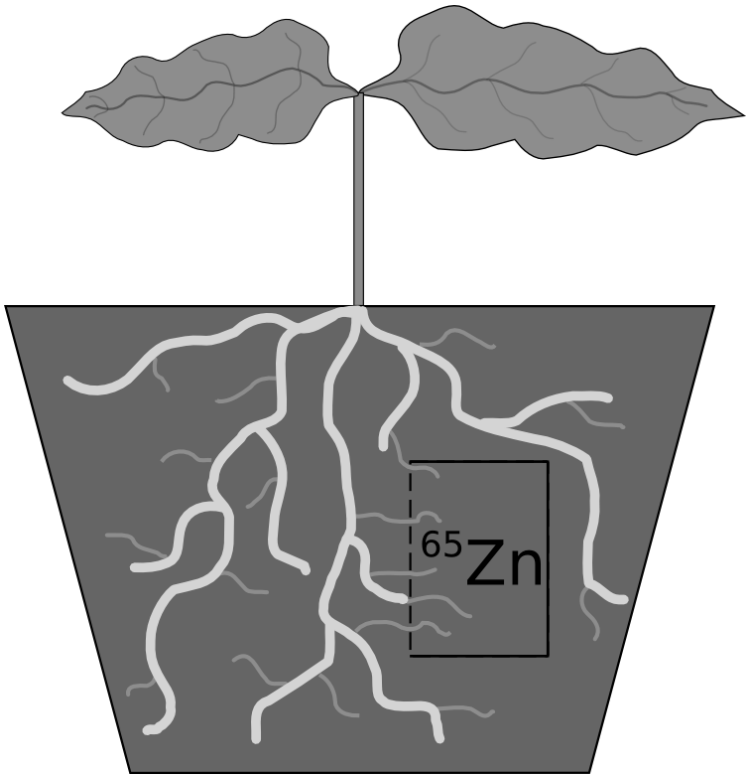

Figure 1

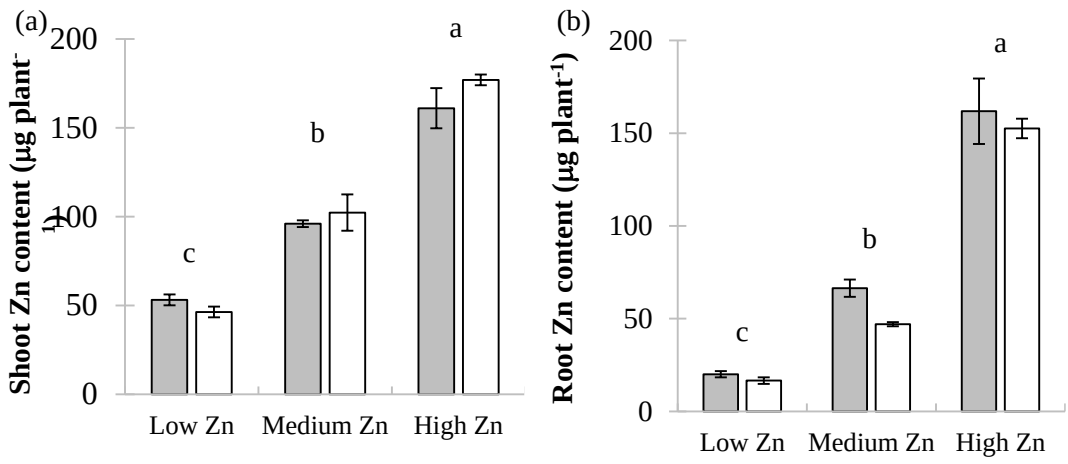
(a)
Shoot Zn content (µg plant-1)
200
150
100
50
0
c
b
a
Low Zn
Medium Zn
High Zn
(b)
Root Zn content (µg plant-1)
200
150
100
50
0
c
b
a
Low Zn
Medium Zn
High Zn

Figure 2

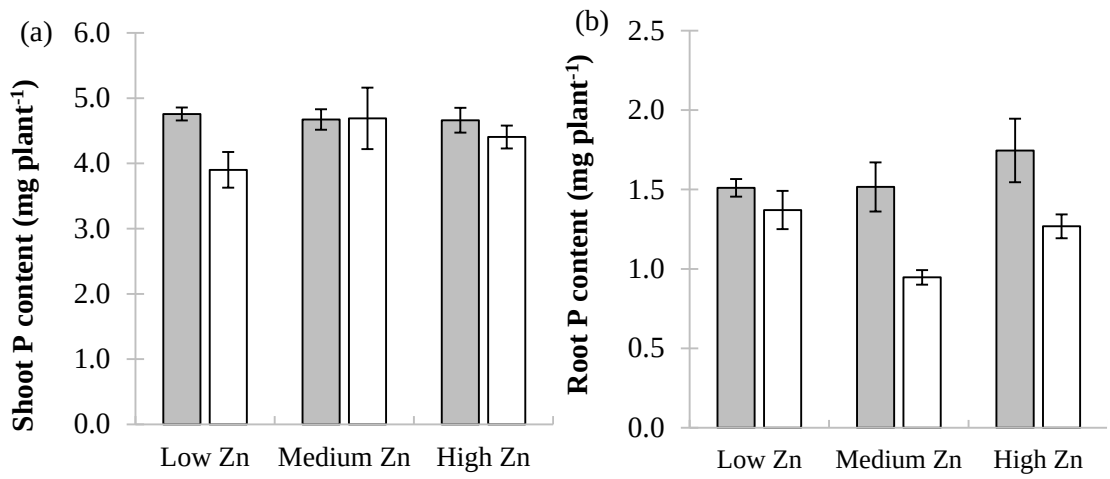
(a)
Shoot P content (mg plant-1)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Low Zn
Medium Zn
High Zn
(b)
Root P content (mg plant-1)
2.5
2.0
1.5
1.0
0.5
0.0
Low Zn
Medium Zn
High Zn

Figure 3

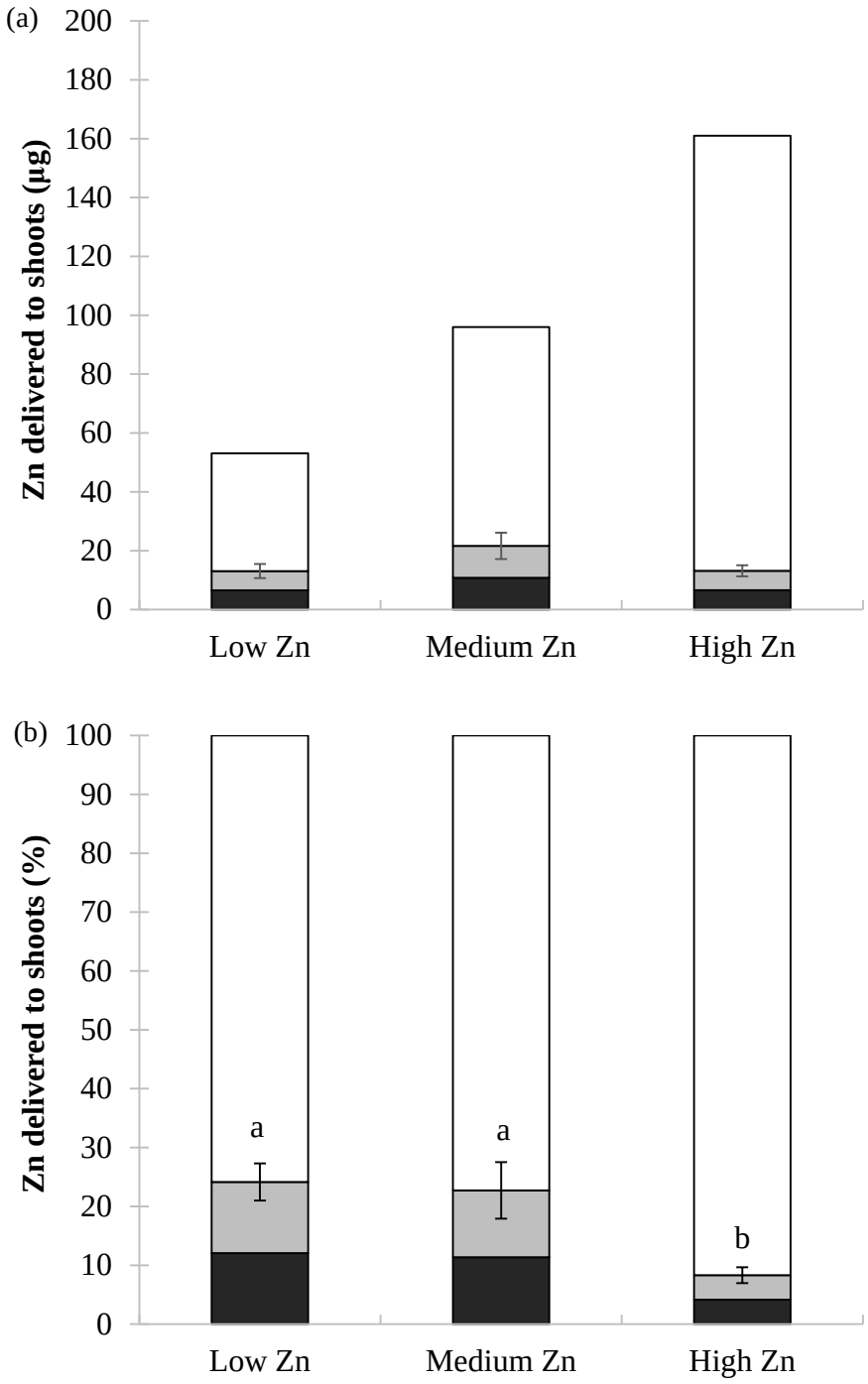
(a)
Zn delivered to shoots (µg)
200
180
160
140
120
100
80
60
40
20
0
Low Zn
Medium Zn
High Zn
(b)
Zn delivered to shoots (%)
100
90
80
70
60
50
40
30
20
10
0
a
a
b
Low Zn
Medium Zn
High Zn

Figure 4

Figure 1. Diagram of experimental set up (not to scale). The hyphal compartment was labelled with ^{65}Zn and capped with nylon mesh that allowed AM fungal external hyphae to enter, while excluding roots (see Methods for further detail).

Figure 2. Shoot (a) and root (b) zinc (Zn) content (μg plant^{-1}) in the mycorrhizal 76R (grey bars) and non-mycorrhizal *rmc* (white bars) tomato (*Solanum lycopersicum*) genotypes, at three soil Zn addition treatments. Values are mean ± standard error, n=5. Means followed by the same letter were not significantly different at the $P \leq 0.05$ level (Tukey's HSD), see Table Appendix 3 for details of ANOVA results.

Figure 3. Shoot (a) and root (b) phosphorus (P) content (mg plant^{-1}) in the mycorrhizal 76R (grey bars) and non-mycorrhizal *rmc* (white bars) tomato (*Solanum lycopersicum*) genotypes, at three soil Zn addition treatments. Values are mean ± standard error, n=5.

Figure 4. Contribution to shoot zinc (Zn) *via* the mycorrhizal pathway of uptake in μg Zn (a) and per cent (%, b) in the mycorrhizal tomato (*Solanum lycopersicum*) genotype (76R), at three soil Zn addition treatments. The segments of each bar indicate AM Zn uptake according to (black) the corrected values of soil specific activity, (black + grey) the raw values of soil specific activity and (white) the remaining Zn (i.e., uptake *via* the DPU). This serves to indicate the range of potential values for % shoot Zn delivered *via* the mycorrhizal pathway of uptake. Values are mean ± standard error, n=5. Means followed by the same letter were not significantly different at the $P \leq 0.05$ level (student's t-test), see Table Appendix 3 for details of ANOVA results.