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Zn Acquisition by Extraradical Hyphae in Arbuscular Mycorrhizal Plant Depending on Zn Nutritional Status of Cucumber (*Cucumis sativus* cv. Baekdadagi)

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ABSTRACT : The contribution of plant nutrition status in arbuscular mycorrhizal (AM) plant to the nutrient acquisition by extraradical hyphae of AM fungi was investigated using cucumber colonized with *Glomus intraradicies* (BEG 110) focusing on the Zn. Compartmentalized pots with separated zones for hyphal growth were used to determine the contribution of extraradical AM hyphae to Zn uptake from hyphal zones. 0.5 μM Zn was supplied into the hyphal zones as nutrient solution (10 mL/day) with a form of $ZnSO_4$. Zn foliar application was made two times for one week before harvest (8 mL/plant). The colonization rate by AM were high in all of Zn treatments. The dry weight of cucumber increased by AM colonization compared to those of non- mycorrhizal counterpart. However, Zn foliar application resulted in no significant difference in dry weight between mycorrhizal- and non-mycorrhizal plant. In addition, the enhancement of Zn content in cucumber shoot by AM colonization were also reduced by Zn foliar application. These results indicate that the interaction between host plant and AM fungus for nutrient uptake might be related to plant nutritional status and nutrient contents. In consequence, higher Zn contents in host plant by foliar application of Zn could restrict the role of extraradical hyphae of AM fungus on the Zn acquisition and transfer from fungus to host plant.

Key words: arbuscular mycorrhiza, Zn nutrition, cucumber, phosphorus.

INTRODUCTION

Mycorrhizas are a widespread mutualistic symbiosis between certain fungal microorganisms and higher plants. About 80~90% of all terrestrial plant species including most vegetables crops are colonized with arbuscular mycorrhiza (AM) fungi and the AM symbiosis has been referred to as the most widespread symbiosis on earth[1)]. The mycorrhizal colonization enhances plant growth by increasing nutrient uptake via an increase in the absorbing surface area, and by mobilizing sparingly available nutrient sources, or by excretion of chelating compounds or ectoenzymes. Most growth enhancement effects by AM colonization are caused by increases in P absorption, particularly from sparingly soluble P sources[2)]. In addition to P, it has been reported that the uptake for both Cu and Zn is increased in AM plants. Li *et al.*[3)] demonstrated increased uptake and translocation of Cu to *Trifolium repens* by AM hyphae. In mycorrhizal plants, the shoot Zn concentrations are usually higher than in non-mycorrhizal plants[4)]. This enhancement can be attributed to uptake and transport of these elements by external hyphae to the host plant[3,5)]. This is primarily due to the extension of hyphae away from the mycorrhizal root into the soil. In addition, many suggestions have been made that fungal hyphae may solubilize certain nutrients in the soil[6-8)]. Cooper and Tinker[9,10)] defined the transport of elements by an AM fungus as the movement of an element from the soil solution into the plant through the fungus via three separate steps:

1. Uptake (from soil solution to fungal cytoplasm, commonly an energy-dependent active process against an ion concentration gradient).
2. Translocation (cytoplasmic streaming within the fungus from the uptake site to the organ and interface toward the host plant).

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3. Transfer (between the fungus and the plant through the inter- or intracellular hyphae colonizing the root cortex, a process that may be linked to symbiotic exchange of C compounds in the opposite direction).

During translocation of nutrients within fungal hyphae, large quantities of nutrients (especially P) and sugar move bidirectionally over long distances in the internal or external fungal hyphae[11].

During transfer of nutrients from fungus to host plant, the requirement of plant to nutrients (e.g. P) could affect the uptake of nutrients by extraradical hyphae of AM fungus. However, it is not well established whether the demand of plant to Zn could affect the interaction between host plant and fungus i.e. acquisition of Zn by extraradical hyphae and transfer of Zn from fungus to host plant.

Therefore, the aim of this study was to evaluate the contribution of plant nutrition status in AM plant to the nutrient acquisition by extraradical hyphae of AM fungus using three compartmentalized pots with root free hyphal part.

MATERIALS AND METHODS

Experimental culture pots

Compartmentalized pot with a central root-free hyphal compartment and two outer root compartments (modification of George *et al.*,[12] were used to investigate direct contribution of hyphae to Zn uptake (Fig. 1). The pots were made of 5 mm acrylic PVC frames (outer root compartments: 8 × 8 × 10 cm; central free hyphal compartments: 5 × 8 × 10 cm). The PVC plates were mounted using PVC glue and the PVC frames for the barriers were mounted using silicon glue. Root compartments were separated from root-free compartments by a nylon mesh (pore size 30 μm) and a wire net (1 mm thickness, 0.1 mm pore size). Consequently, only the hyphae but no roots could grow into outer compartments, and massflow of nutrients from root-free hyphal part to root part could avoid.

Substrate composition and preparation

Sand (0.1~0.5 mm diameter) and horticultural vermiculite was used as substrate with a mixture as ratio of 1:1 (v/v). Vermiculite and sand were prepared by washing with tap water over a 16 mesh sieve, oven-drying for 24 h at 100℃. To eliminate indigenous mycorrhizal fungi, the substrate was autoclaved (121℃, 20 min) for the sterilization and thereafter sand and vermiculite were mixed uniformly as ratio of 1:1 (v/v). For root-free hyphal part, glass beads (1 mm diameter) were used to collect fungal hyphae at harvest after autoclaving (121℃, 20 min). Nutrients addition to substrate were

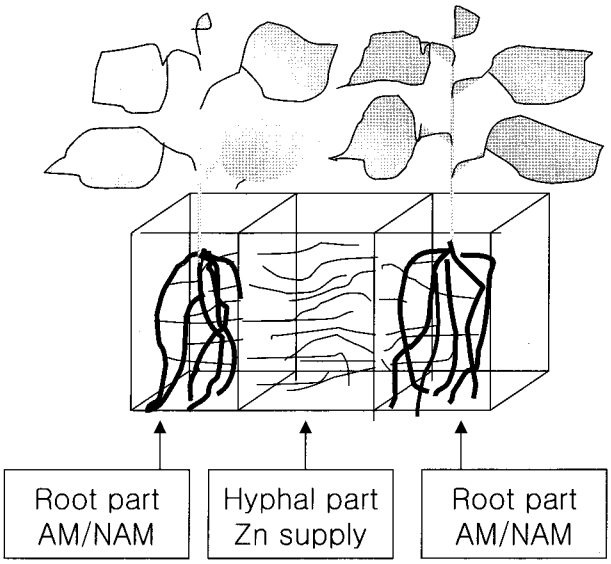


Fig. 1. Three compartmentalized pots separated with nylon mesh (pore size 30 μm) and wire net (1 mm thickness, 0.1 mm pore size) between root and root-free hyphal compartments.

made at the beginning of the experiment. Nutrients were supplied in the following components and concentration for root [kg/soil]: 150 mg N ($Ca(NO_3)_2$, 20 mg P $Ca(H_2PO_4)_2$, 150 mg K (K_2SO_4), 50 mg Mg ($MgSO_4$), 50 mg Fe (Fe-EDTA), 20 mg Mn ($MnSO_4$), 0.2 mg Mo ($(NH_4)_6Mo_7O_{24}$).

Plant and fungus culture

Cucumber (*Cucumis sativus* cv. Baekdadagi) were planted with or without the mycorrhizal fungus *Glomus intraradicies* isolate (BEG 110). The seeds were sterilized with 10% H_2O_2 for 10 min and then pre-germinated in saturated $CaSO_4$ and sown directly into the pot. The bulk density of substrate was 1.3 g/cm^3. After germination, plants were thinned to one plant for cucumber. For colonization with AM fungi, an inoculum was added to 10% of soil weight (w/w). The mycorrhizal inoculum consisted of the same substrate as used in the experiment and contained AM spores, hyphae, and white clover root pieces colonized. Plants were grown in a climate controlled chamber (min/max temperature of 22/25℃; light intensity of approximately 200 μ mol/m^2/s). Pots were watered one or two times per day depending on plant growth with distilled water to maintain a soil water content equivalent to 20% weight of substrate (w/w) (determined gravimetrically). After eight weeks, shoots and roots were harvested for analysis of dry weight and determination of nutrient concentrations. 1 cm long root fragments (0.25 g, three replicates) were sampled from fresh root before drying.

Zn treatment

0.5 μM $ZnSO_4$ was supplied only for the root-free hyphal

compartment as a solution (10 mL/day) from the beginning of experiment. After 4 weeks, root-free hyphal part was washed with distilled water several times. One week before Zn foliar application, Zn-starved condition was induced by intermittence of Zn supply in hyphal growing zones. Thereafter, Zn foliar application was made three days interval, two times for a week (8 mL/plant).

Mycorrhizal root colonization

The percentage of root length colonized by mycorrhizal fungi was determined on roots stained in trypan blue[13] using the gridline-intersect method[14].

Nutrient analysis

After harvesting, plants shoot and roots were dried at 60℃ for three days and pulverized. Pulverized shoot and roots material (0.25 g) were dry ashed at 500℃ for 4 h, after moistened with H_2O and 1:3 HNO_3 (v/v), which was evaporated off to split SiO_2 from other compounds. Samples were then dissolved in 1:30 (v/v) HCl and boiled for 2 min (to convert metaphosphates and pyrophosphates to orthophosphates) before being made up to volume (25 mL) with doubly distilled water. The digested solution was filtrated with filter paper (Watman No. 2) and Zn concentration was measured with ICP.

Phosphorus concentration was determined in the same solution using the molybdenum blue assay[15].

Statistics

Four replicate pots per treatment with one plant were used for cucumber. Student's *t*-tests were applied to determine differences in the presence or absence of mycorrhizal fungi.

RESULTS AND DISCUSSION

Mycorrhizal root colonization

High level of colonization (above 50%) was observed for cucumber plants irrespective of Zn foliar application applied to the hyphal compartment (Table 1). No colonization was observed in non-inoculated plants. Colonization pattern of cucumber root was shown in Fig. 2.

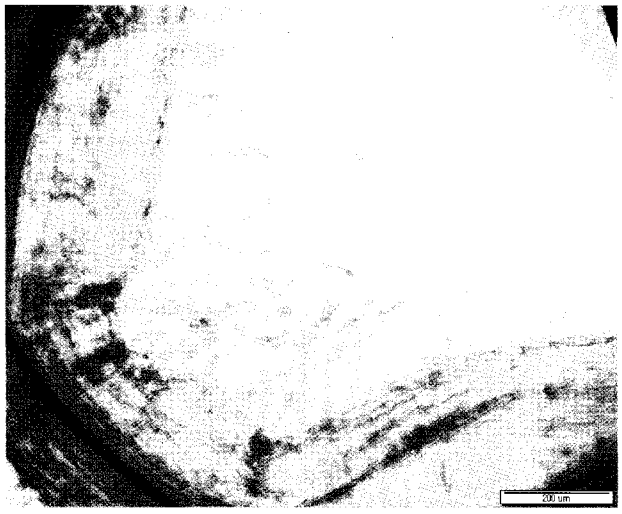
Growth response

It is well established that a mycorrhizal colonization can improve plant nutrient uptake of P[16-19], Zn[5,20] and Cu[3,20] due to improved uptake of these elements by the extraradical mycorrhizal hyphae. We could also find that mycorrhizal cucumber plants showed increased shoot growth compared to non- mycorrhizal plants (Table 1). It could be attributed the role of extraradical hyphae of

Table 1. Root colonization and shoot and roots dry weight of non-mycorrhzial (NAM) and mycorrhizal (AM) cucumber grown at Zn supply to the root-free hyphal compartments. Different letters indicate statistical difference between NAM and AM (*P*< *0.05, Student's t-test*). Values are means ± SE of four replicates

Treatment	Infection rate (%)	Shoot dry weight (g)	Root dry weight (g)	Shoot /Root (ratio)	Mycorrhizal dependency
Control					
AM	53	2.60 ± 0.27a	0.32 ± 0.05a	8.38	141.7
NAM	0	1.85 ± 0.08b	0.21 ± 0.01b	9.07	100
Zn application					
AM	57	2.34 ± 0.21a	0.28 ± 0.05a	9.33	104.7
NAM	0	2.25 ± 0.18a	0.20 ± 0.03a	8.81	100

AM



NAM

Fig. 2. Colonization patterns of mycorrhizal (AM) cucumber root colonized with *G. intraradicies* (BEG 110) compared to that of non-mycorrhizal (NAM) cucumber root.

AM fungus. Zn supply to external hyphae enhanced shoot and roots growth in mycorrhizal cucumber plants (Table 1). To investigate the effect of plant nutritional status on the interaction between fungus and host plant for Zn uptake, we tried to make sufficient Zn nutritional status for cucumber using Zn foliar application. Interestingly, Zn foliar application reduced the mycorrhizal effect on the plant growth, which showed improved dry weight in cucumber. Mycorrhizal plant had no significant difference in shoot and roots dry weight compared to those of non-mycorrhizal counterpart (Table 1). This tendency could be verified by the result of mycorrhizal dependency of cucumber plant. Zn foliar application resulted in lower mycorrhizal dependency compared to that of control without Zn foliar application (Table 1). Therefore, sufficient Zn nutrition of host plant could result in restriction of hyphal activity of symbiont AM fungus for nutrient acquisition and transfer and, consequently, reduce mycorrhizal dependency of cucumber.

P concentrations and contents

Mycorrhizal colonization resulted in an obvious increase in P concentration of roots in cucumber (Fig. 3). In contrast, shoot P concentration did not show a significant difference between mycorrhizal- and non-mycorrhizal plants (Fig. 3) indicating a dilution effect by enhanced shoot growth by AM colonization. Due to enhanced growth response in AM plant, the dilution effect on the nutrient concentration had been reported by many researchers[12,18]. Therefore, mycorrhizal effect on P uptake in plant could be found more in P contents compared to P concentrations. Shoot and roots P contents of cucumber were increased obviously by AM colonizaiton (Fig. 3). However, Zn foliar application reduced this mycorrhizal effect on P contents resulting in small extent of difference between mycorrhizal and non-mycorrhizal cucumber indicating that there might be a link between P and Zn nutrition in mycorrhizal plant (Fig. 3).

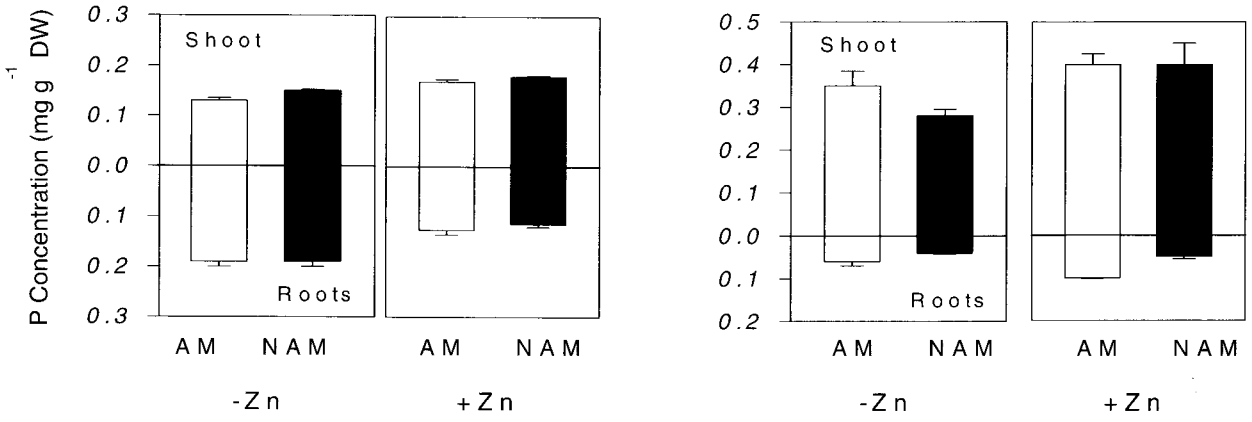


Fig. 3. P concentrations and contents of non-mycorrhzial (NAM) and mycorrhizal (AM) cucumber shoot and roots grown at Zn supply to the root-free hyphal compartments depending on Zn foliar application. Values are means±SE of four replicates. -Zn, without Zn foliar application; +Zn, with Zn foliar application.

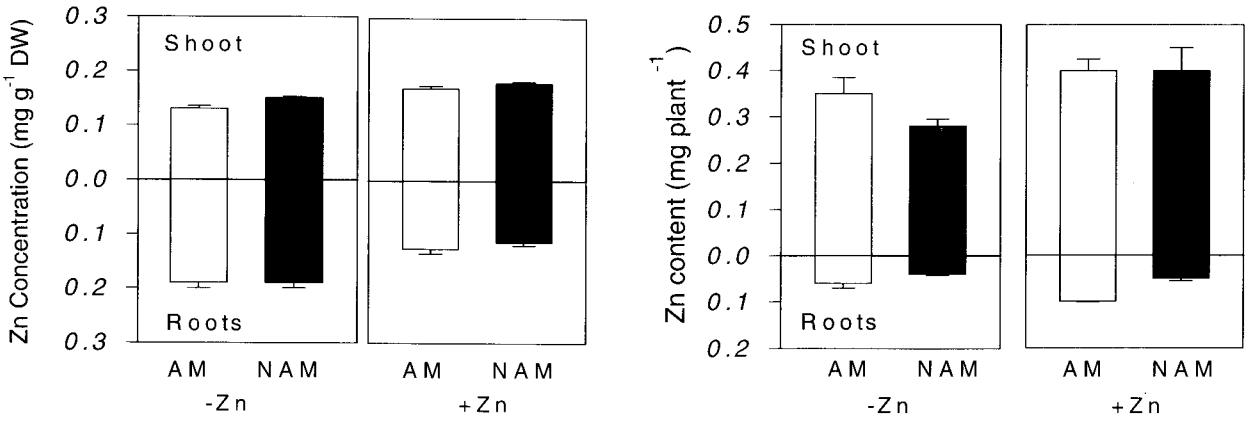


Fig. 4. Zn concentrations and contents of non-mycorrhzial (NAM) and mycorrhizal (AM) cucumber shoots and roots grown at Zn supply to the root-free hyphal compartments. Values are means±SE of four replicates. -Zn, without Zn foliar application; +Zn, with Zn foliar application.

Zn concentrations and content

The shoot and roots Zn concentrations in mycorrihzal cucumber plants did not show any significant difference by Zn supply into the fungal hyphal part compared those of the non-mycorrhizal plants (Fig. 4). It was inconsistent of other reports which showed higher Zn shoot and roots concentration in red clover[3,21,22]. It could be attributed to the dilution effect by enhanced plant growth in AM plant and insufficient growth of fungal mycelium due to short experimental periods (6 weeks). Under this condition, small amount of fungal hyphae in mycorrhizal cucumber entered into the hyphal part containing Zn supplied. The abilities of the AM fungal hyphae are to grow in root-free part, to absorb and transport Zn to the host plants[21]. Nevertheless, Zn content of shoot was increased by Zn supply into AM fungal hyphae. However, Zn foliar application resulted in no significant difference in shoot Zn content of mycorrhizal plant compared to that of non-mycorrhizal counterpart. Interestingly, Zn content of roots was increased by Zn foliar application (Fig. 4). Compared to adequate range of Zn concentration (35~80 ppm) in cucumber[23], Zn concentrations in cucumber shoot were sufficient for plant growth (Fig. 4). Nevertheless, higher Zn concentration by Zn foliar application reduced the mycorrhizal effect on the improvement of Zn content in cucumber compared to those of non-mycorrhizal counterpart.

The relationship of P and Zn

The concentration ratio of P/Zn was decreased in AM shoot, while increased in AM root. There were some reports that plant Zn concentration was positively related to AM colonization, but negatively related to P fertilizer[4,24]. The higher P nutritional status of AM plant by higher P fertilization might result in decrease of AM fungal activity of extraradical hyphae on the nutrient uptake and as a consequence, might result in decrease of Zn acquisition by extraradical hyphae of AM fungus. In P and Zn nutritional interaction in plant, high P physiological availability could result in decrease of Zn efficiency in plant[25]. Therefore, considering these results, decrease of P/Zn ratio in mycorrhizal cucumber shoot and increase in mycorrhizal cucumber root indicated that higher translocation of Zn from root to shoot in AM cucumber compared to that of P. However, Zn foliar application resulted in reduced differences in P/Zn ratio between AM and non-mycorrhizal counterpart (Fig. 5).

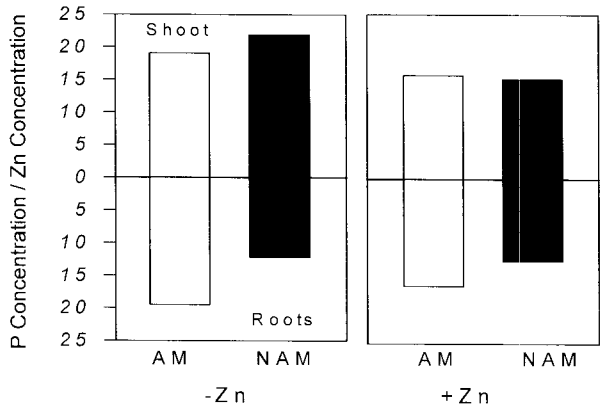


Fig. 5. The relationship of P and Zn concentrations of non-mycorrhzial (NAM)and mycorrhizal (AM) cucumber shoot and roots grown at Zn supply to the root-free hyphal compartments. -Zn, without Zn foliar application; +Zn, with Zn foliar application.

In conclusion, the interaction between host plant and AM fungus for Zn uptake might be related to plant Zn nutritional status, in consequence, higher Zn nutrition of host plant by Zn foliar application could restrict the role of extraradical hyphae of AM fungus on the Zn acquisition and transfer from fungus to host plant in plant-fungus interaction.

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