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Effects of arbuscular Mycorrhizal fungi (AMF) inoculums on cucumber seedlings

Abstract

To study the effects of AMF (arbuscular mycorrhizal fungi) inoculums on seedling growth of cucumber, vegetable seeds were inoculated with two kinds of AMF inoculums BF (mycorrhiza for plants) and VT (mycorrhiza for vegetables) from Czech Republic during sowing in trays in greenhouse. The root AMF infection rates in seedlings were 49.30% and 37.65% respectively after 35d growth. The plant height, stem diameter, fresh and dry weight of those seedlings inoculated with BF and VT were higher than that in CK. Compared with the CK, VT inoculation significantly improved the root activity, chlorophyll content and photosynthetic rate of cucumber seedlings. Comprehensive analysis showed that VT could promote seedling growth of protected cucumber.

Keywords: cucumber, arbuscular mycorrhizal fungi (AMF) inoculums, Seedling growth, Photosynthetic physiology

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Introduction

Arbuscular mycorrhizal fungi (AMF) is a kind of soil microbes that is symbiotic with plant roots, which has a variety of functions as follows: promoting plant growth, improving Rhizosphere environment, improving plant absorption of beneficial nutrients P, K, Ca, Cu and Fe by extending hyphae, increasing plant resistance to adverse environment, defending harms of other harmful microbes on plant, and improving yield and quality of crops.[1] Although the symbiotic effect between single AMF strain and cucumber has been reported[2–5] different AMF strains can influence the metabolic process of plant through different modes and approaches after symbiosis. The inoculum composed of different strains can be used for seedling inoculation, which is featured by simple operation, low cost and low volume.[6] There is no commodity mixture inoculum suitable for domestic cucumber seedling in production. Two AMF mixture inoculums introduced from Czech Republic were applied in cucumber nursery. Seedling growth and photosynthetic physiological indexes of cucumber were measured, in order to screen commodity AMF mixture inoculums suitable for cucumber nursery in China.

Materials and methods

Materials

The cucumber variety used in the test was Zhongnong 16, breed by Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences; special commodity compound inoculums for vegetables, VT (mycorrhiza inoculum for vegetable, VT in abbreviation, the mixed strain of *Glomus claroideom*, *G. funneliformis*, *G. diversispora* and *G. rhizophagus*, produced by Czech Symbiom Coopearation), and plant universal inoculum BF (mycorrhiza inoculum for plants, in abbreviation, the mixed strain of *G. intradices* BEG140, *G. microageregatum* BEG, *G. claroideum* BEG 210), were provided by professor Vosatka Miroslav at Institute of Botany, Czech Academy of Sciences. The inoculums contained 800-1000 mycorrhizal spores per gram.

Experimental methods

The trial was performed in the solar greenhouse of Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences in the spring of 2015. Three treatments were designed in the test, including VT inoculation, BF inoculation and CK (non-inoculation), and each treatment was repeated three times. Cucumber seeds were soaked in warm water at 55°C for 10 min, then soaked in normal temperature water for 8h, and finally accelerated germination in an incubator at 30°C. Nursery substrates were prepared with peat and vermiculite at the proportion of 2V:1V; the substrates were processed in air dry oven at 160°C for 2h, and the process was repeated one more time; the substrates were preserved for later use when cooled naturally. On February 4, germinated cucumber seeds were sown in trays and inoculated with inoculums. The 50-hole trays were used for nursery, which were wiped with 70% alcohol and dried; 10% (160g) mycorrhizal inoculums were applied in each treatment; inoculums were mixed with substrates and loaded into trays, 1600g each tray. Equal volume of sterilized inoculums was applied in CK, to ensure that trace elements were consistent.

Measurement of items

Plant height (height from cotyledon to growing point) and stem diameter (the thickness of cotyledon section parallel to cotyledon expanding direction) of seedlings were measured at 32, 39, 46 and 53 d after seeding, respectively. Ten seedlings were sampled at 36 d after seeding; the aerial part and underground part of seedlings were taken, rinsed with tap water to remove surface debris, then rinsed with deionized water; after removed moisture, the fresh weight was measured, and the average fresh weight per plant was obtained; afterwards, the samples were deactivated at 105°C for 15 min, and dried at 75°C to constant weight; the samples were weighed, to get average dry weight per plant. At 60 d post inoculation, the fifth functional leaves at the same position of cucumber plants were selected from each treatment; the photosynthetic indexes, such as photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci) and transpiration rate (Tr), were measured with LI-6400 photosynthetic apparatus; chlorophyll content was determined using acetone method; root activity was determined using TTC method.[7] Root samples were collected at 35 d after seeding, to determine infection rate, and mycorrhizal infection rate was calculated according to frequency standard method.[8]

Data processing

Data were processed using Excel software and DPS v7.05 software, and significance of difference was analyzed using Duncan's new multiple range method.

Results and analysis

Effect of different AMF inoculums inoculation on growth of cucumber seedlings

As shown in Figure 1 & 2, the plant height and stem diameter of cucumber seedlings inoculated with VT and BF inoculums were increased compared with CK; VT inoculation received the best effort, which had significant difference with the other two treatments. The plant height and stem diameter of cucumber seedlings inoculated with VT inoculum were increased by 108.0% and 31.7% compared with CK, respectively.

Effect of different AMF inoculums inoculation on photosynthetic characteristics of cucumber seedlings

The net Pn, Gs and Tr of cucumber seedlings in BF inoculation treatment were the highest, which had significant difference with those in CK and VT inoculation treatment (Table 1). Among them, the net Pn were increased by 34.43% and 9.54% compared to those in CK and VT inoculation treatment, respectively. The net Pn in VT inoculation treatment was significantly higher than that in CK, but the other indicators in VT inoculation treatment were significantly lower than that in CK.

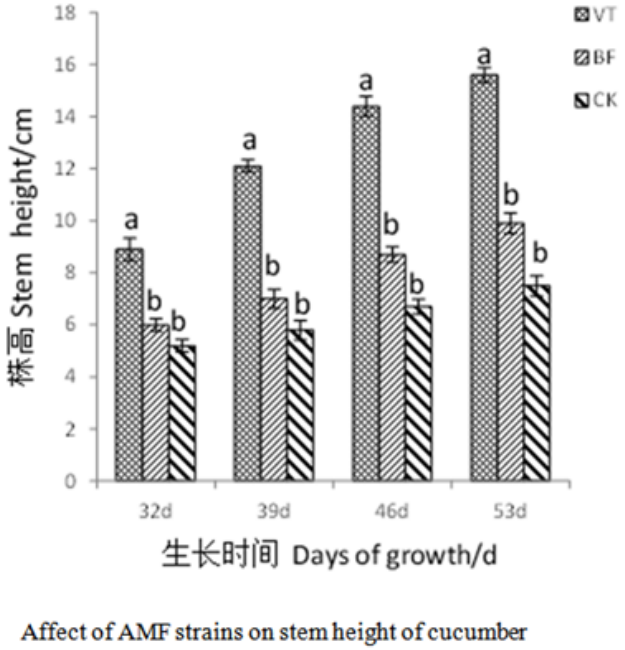

Figure 1 Effect of different AMF inoculums on plant height and stem diameter of cucumber seedlings

Note: Different lowercase letters above the cylinder represent significant difference among treatments (P<0.05). Plant height //cm, Stem diameter //mm, Growth time//d,VT inoculation, BT inoculation, CK]

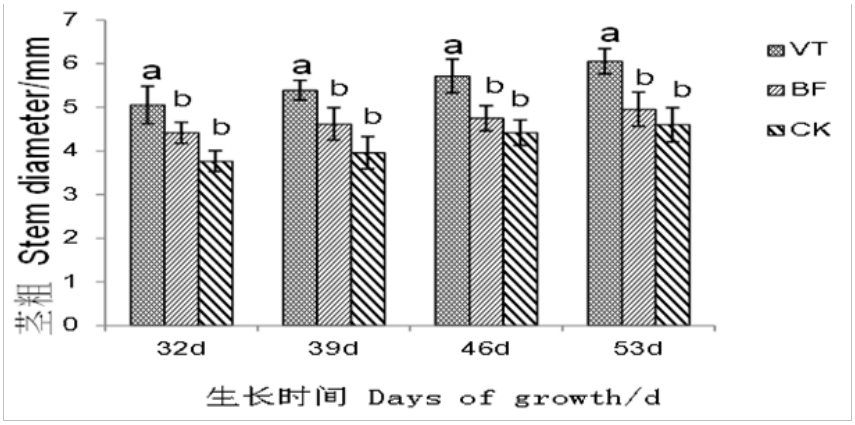

Figure 2 Effect of AMF strains on stem diameter of cucumber

Table 1 Effect of different AMF inoculums inoculation on photosynthetic characteristics of cucumber seedlings

Treatment	Net photosynthetic rate (Pn) //μmol/(m^2 s)	stomatal conductance (Gs) //mmol/(m^2 s)	Intercellular CO_2 concentration (Ci) //μL/L	Transpiration rate (Tr)//mmol/(m^2 s)
VT inoculation	10.48 b	0.22 c	322.88 b	3.25 c
BF inoculation	11.48 a	0.36 a	349.33 a	5.04 a
CK	8.54 c	0.24 b	346.79 a	3.65 b

Note: Different lowercase letters in the same column represent significant difference at 0.05 level; the same as below.

Effect of different AMF inoculums inoculation on chlorophyll content and root activity of cucumber seedlings

Compared with CK, the chlorophyll content and root activity of cucumber seedlings inoculated with AMF inoculums increased obviously; the chlorophyll content and root activity of leaves inoculated with VT inoculums were increased by 19.43% and 50.17% compared with CK, respectively. Although the chlorophyll content of seedlings inoculated with BF inoculum was slightly increased compared with CK, the difference was not significant; the root activity of seedlings inoculated with BF inoculums was increased by 18.94% compared with CK (Table 2).

Table 2 Effect of different AMF inoculums inoculation on chlorophyll content and root activity of cucumber seedlings

Treatment	Chlorophyll a //mg/g	Chlorophyll b//mg/g	Total chlorophyll //mg/g	Root activity //μg/(g·h) FW
VT inoculation	16.72 a	5.43a	22.13 a	56.84 a
BF inoculation	15.06 b	4.78ab	19.85 b	45.02 b
CK	14.02 b	4.51 b	18.53 b	37.85 c

Effect of different AMF inoculums inoculation on matter accumulation and mycorrhizal infection rate of cucumber seedlings

As shown in Table 3, the growth potential of cucumber seedlings inoculated with VT and BT were better than that in CK; the average fresh weight of aerial part, fresh weight of underground part, dry weight of aerial part and dry weight of underground part in VT inoculation treatment were increased by 85.83%, 72.06%, 43.26% and 112.05% respectively compared with CK, and the difference reached significant level. The average fresh weight of aerial part, fresh weight of underground part, dry weight of aerial part and dry weight of underground part in BF inoculation treatment were increased by 22.05%, 33.82%, 9.30% and 15.66% respectively compared with CK. The fresh weight root/shoot ratio of seedlings inoculated with VT inoculums had no significant difference with the other two treatments, while dry weight root/shoot ratio was significantly higher than the other two treatments. In conclusion, VT inoculation had the best effect on promoting plant growth and dry matter accumulation of cucumber, which was the mycorrhizal inoculum suitable for cucumber.

Table 3 Effect of different AMF inoculums inoculation on matter accumulation and mycorrhizal infection rate of cucumber seedlings

Treatment	Fresh weight //g/plant		Dry weight //g/plant		Root/shoot ratio		Mycorrhizal infection rate //%
	Aerial part	Underground part	Aerial part	Underground part	Fresh weight	Dry weight	
VT inoculation	4.72 a	2.34 a	0.616 a	0.176 a	0.495 ab	0.286 a	49.3
BF inoculation	3.10 b	1.82ab	0.470 b	0.096 a	0.586 a	0.204 b	37.65
CK	2.54 b	1.36 b	0.430 b	0.083 b	0.535 a	0.193 b	-

Conclusions and discussion

AMF symbionts can accelerate root growth and nutrient recycling of plants. In root system development process of plants, good mycorrhizal structure formed with suitable mycorrhizal fungi will improve yield, and the effect is greater especially in drought thin soil.[1] Symbiotic effect of single AMF strain and cucumber has been reported. Wang et al.,[3] found that cucumber seedlings inoculated with VA inoculum had stronger growth ability than ordinary seedlings, and VA inoculation increased cucumber yield remarkably. Ren et al.,[9] found that after inoculation of AMF strain *Glomus mosseae* at nursery stage, the growth potential of cucumber seedlings was enhanced, and yield and quality was improved, such as declined nitrate content, increased VC and soluble solid content. Previous studies also pointed out that AMF inoculation enhanced the resistance of cucumber seedlings to damping-off,[10] improved the resistance to low temperature at seedling stage,[11] greatly increased mycorrhizal infection rate of cucumber seedling, strengthened nutrient absorption ability of seedlings, and improved seedling quality.[12–15]

Inoculation of special vegetable mycorrhizal inoculum VT at seedling stage significantly increased chlorophyll content, photosynthetic rate and root activity of cucumber seedling, enhanced growth potential, plant height and stem diameter, and significantly improved dry weight and fresh weight of aerial part and underground part. Different AMF strains led to robust growth, more developed root system, and higher root activity by infecting root system of protected cucumber seedling, which promoted absorption of root system to water and nutrients, thus promoting the growth of plant. Researchers at Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences also received good effect in VT inoculums inoculation at seedling stage of protected tomato[1]. Currently, inoculation of single strain has not been widely applied in production due to unstable effects, while propagation of AMF depends on specialized production of host plant, and this is an issue to be settled urgently in domestic production of inoculums. Special vegetable VT inoculums manufactured by Czech Symbiom Cooperation realizes specialized and standardized production, with good promotion prospect.

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Conflicts of interest

The authors declared there is no conflicts of interest.

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