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Effect of Vesicular-Arbuscular Mycorrhizae on the Growth of Bell Pepper and Corn Seedlings

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고추와 옥수수 실생의 생장에 미치는 균근의 효과

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ABSTRACT

Effects of mycorrhizal infection on the growth of bell pepper (*Capsicum annuum*) and corn (*Zea mays*) seedlings have been studied by comparing plants grown in sterilized soil/sand mixtures to plants grown in sterilized soil/sand mixtures with topping the original non-sterile field soil. The original non-sterile field soil, which were taken from the bell pepper field, contained a high level of endomycorrhizal spores. After seven weeks, the shoot height of inoculated plants was increased by 110% in bell pepper, and 90% in corn compared with the control plants. The average above-ground biomass of inoculated plant was increased by 88% in bell pepper and 71% in corn compared with the control plants. The shoot/root ratios in bell pepper and corn were 2.7 and 1.8 for the control plants, and 4.3 and 2.7 for the treatment plants, respectively. Phosphorus level in inoculated plant was higher than that of the control plant. However, nitrogen contents were similar between the control and the treatment plants. The control plants did not form vesicular-arbuscular mycorrhizae during the experimental period.

INTRODUCTION

Mycorrhizae are symbiotic associations, between fungi and the roots of higher plants, in which both members normally benefit from the association. Within the endomycorrhizae, those produced by nonseptate fungi are referred to as vesicular-arbuscualr mycorrhiza (VAM) (Safir, 1980). Vesicular-arbuscular mycorrhizal fungi are ubiquitous in soils throughout the world (Abbott and Robson, 1984). These fungi form mycorrhizae with a majority of plant species and show little host specificity (Gerdemann, 1968; Mosse *et al.*, 1981): Vesicular-arbuscular mycorrhizae improve plant nutrition and vigor, especially in nutrient deficient soils (Mosse, 1973; Gerdemann, 1975; Mosse and Tinker, 1980; Hass *et al.*, 1986). Plants with vesicular-arbuscular mycorrhizae frequently exhibit increased growth in comparison to non-mycorrhizal controls. This growth response is most often attributed to an increase in the uptake of phosphate (Gray and Gerdemann, 1969; Mosse, 1973; Sanders and Tinker, 1973; Rhodes and Gerdemann, 1975). Studies on phosphorus uptake

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from isotopically labeled media have shown that the external fungal hyphae are able to absorb phosphorus directly from the soluble phoshorus pools in the soil and translocate it to the host root (Rhodes and Gerdemann, 1980; Cooper and Tinker, 1978, 1981). Besides the generally accepted phenomenon of improved growth and nutritional status, vesicular-arbuscular mycorrhizae also can improve the water relations of the host plant (Christensen and Allen, 1979, 1980; Safir and Nelson, 1981), and can control the root pathogens (Trappe, 1977). These possible role of mycorrhizae had attracted many workers from different disciplines (Powell and Bagyaraj, 1984). Because of the current inability to grow vesicular-arbuscular mycorrhizal fungi in pure culture, however, much of the eco-physiological properties of versicular-arbuscular mycorrhizae are still uncovered (Menge, 1984). Another problem in the study of mycorrhizal symbiotic association is the difficulty of obtaining valid controls in which the association has been broken (Smith and Smith, 1981).

In our studies of the effects of vesicular-arbuscular mycorrhizal inoculation on the growth and phosphorus uptake of bell pepper and corn seedlings, we compared plants grown in sterillized soil/sand mixture (control), and sterilized soil/sand mixture with topping the original non-sterile field soil.

MATERIALS AND METHODS

Pot Preparation

The soil which was taken from the bell pepper field was sterilized by autoclaving in trays at 120°C for 30 min. Several days before seedlings were planted, sterilized soil was mixed with sand, which had been washed and steamed. The soil/sand mixture was 50% soil: 50% sand. In each pot diameter 11 cm, height 13 cm, one kg of mixed soil was added. The initial nitrogen and available phosphorus contents in the experimental soil mixture were 0.3 mgN/g and 0.04 mg P/g, respectively.

The pots were divided into two groups, control and treatment. The original non-sterile field soil was applied in the treatment pots, about 5 mm thickness on the top of the pot, to reintroduce mycorrhizal propagules. All pots were then wetted with deionized water. Five germinated seeds, germinated from surface-sterilized seeds, were planted in each pot. All the pots were placed in the open air where protected from rain water. During the experimental period, from July to August, the pots were applied with 200 ml of distilled water at every two days.

Spore Enumeration

Spores were recovered from the treatment pots by a modification of the flotation-adhesion technique (Sutton and Barron, 1972). 10 g of soil sample was placed in 100 ml of water in a 100 ml graduated cylinder and shaken for 20 sec. The supernatant was immediately poured from the cylinder into a separatory funnel. The extraction procedure was repeated twice. The contents of separatory funnel and washing of its inner surface were filtered through Whatman #4 filter paper with Buchner funnel. Individual spores were picked from the filter paper with the aid of a dissecting microscope at 30X. The spores at the permanent slide were observed and measured with compound microscope at 100-400X. The identifications of spores were made by the description of Schenck and Perez (1988), and Hall and Abbott's (1981) slide guide.

Plant Sampling and Treatment

Plant sampling was begun three weeks later after planting in the pots. Ten plants were sampled at every week in each control and treatment pots. Plants were divided into above-ground and below-ground portion after washing with tap water. Individual plant height and dry weight (after drying at 60°C oven for 48 h) were measured. Subsamples of root systems were stained after collection by the method of Philips and Hayman (1970), and stained roots were examined with a compound microscope for the observation of mycorrhizae formation. Total nitrogen was determined by microkjeldahl method (Wilde *et al.*, 1979). Plant phosphorus was determined by a wet digestion method (Allen *et al.*, 1974).

RESULTS AND DISCUSSION

Growth Responses

The overall plant growth was quite different betwen the control (non-mycorrhizal) and the treatment (mycorrhizal) plants. Differences of plant height between the control and the treatment plants began 4 weeks in bell pepper and 5 weeks in corn after planting (Fig. 1). Besides the plant height, the treatment plants were more vigorous than the control plants. At the end of the experiment, average weight of individual plant in treated bell pepper and corn was 0.15g and 0.82g, respec-

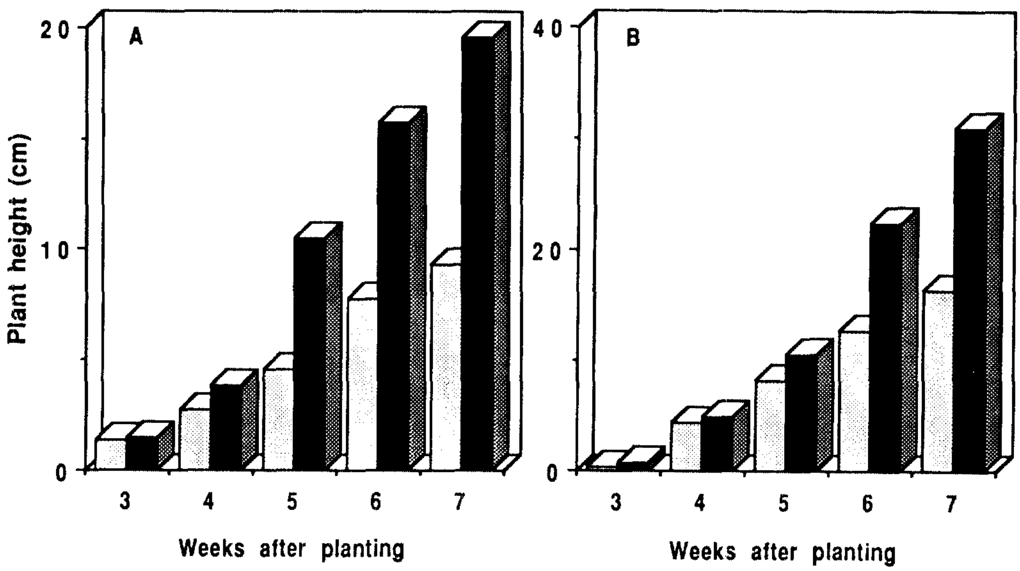

Fig. 1. Average plant height of the control (dotted) and treatment (black) in bell pepper (A) and corn (B).

Table 1. Growth responses of bell pepper and corn in the control (CON) and treatment (TRT) after seven weeks (mean ± SE)

	Bell pepper		Corn	
	CON	TRT	CON	TRT
Plant Height (cm)	9.3 ±0.43***	19.5±0.54	16.3±1.68***	31.0±2.43
Biomass (g)	0.08±0.01***	0.48±0.03	0.48±0.07***	0.82±0.07
Shoot/Root	2.7	4.3	1.8	2.7

***$p<0.001$

tively (Table 1). On the other hand, that of the control was 0.08 g and 0.48 g, respectively. The shoot/root ratios in bell pepper and corn were 2.7 and 1.8 for the control plants, and 4.3 and 2.7 for the treated plants, respectively. The higher shoot/root ratio in the treated plants indicates that the root growth is comparatively fast in the control plants. A problem common to the study of mycorrhizal symbiotic associations is the difficulty of obtaining valid controls in which the association has been broken (Smith and Smith, 1981). The autoclaving process may alter the physical structure of the original soil, which may cause difficulty in root system expansion. We eliminated this problem by using the same sterillized soil/sand mixture in both the control and the treatment pots. The soil/sand mixture showed no prevention of root system expansion in the control pots. Therefore, the higher shoot/root ratio in the control plants indicates that the more root systems are required to take up necessary inorganic nutrients.

Spore Identification and VAM Formation in Root

The following fungal species were identified in the treated pot soil.

1) *Acaulospora bireticulata* Rothwell and Trappe (1979), Mycotaxon 8:471-475. Azygospores of this species were observed in the treatment pot soil of corn and bell pepper. The prominant feature of this spore was surface morphology ornamented with a polygonal reticulum.

2) *Acaulospora spinosa* Walker and Trappe (1981), Mycotaxon 12:515-521. Azygospore formed singly in soil, usually globose, yellowish brown to dark brown.

3) *Gigaspora decipiens* Hall and Abbott (1984) Trans. Br. Mycol. Soc. 83:203-208. Spores are globose, yellow or light brown.

4) *Glomus deserticola* Trappe, Bloss and Menge (1984) Mycotaxon 20:123-127. Spores borne singly or in loose fascicles in soil. Reddish brown with a single wall. Attached hypha is usually funnel shaped.

5) *Scutellospora persica* (Koske and Walker) Walker and Sanders (1985) Mycologia 77:702-720. Spores formed singly in the soil terminally on a bulbous suspensor-like cell. Globose or ellipsoid. Orange or brownish orange.

Until the end of the experiment, bell pepper and corn plants in the control plot did not show VAM formation. However, those in the treated plants showed VAM formation. The VAM formation began to appear from 3 weeks after planting. At the end of the experiment, we can observe vesicles and longitudinally running hyphae in root cortex (Plate 1).

N and P Contents in Plant Tissue

The nitrogen contents of bell pepper and corn were quite similar between the control and the treatment plants. In the 3 weeks after planting, nitrogen content of the bell pepper in the control and the treatment was 41 and 39 mg N/g, respectively. During the growth, there was no significant difference in nitrogen contents between the control and the treatment. After 7 weeks, nitrogen contents decreased to 20 mg N/g in both of the control and the treatment (Fig. 2). The result of nitrogen of the corn in the control and the treatment was quite similar with the bell pepper.

The phosphorus contents in plant tissue decresed as the plant grow. In the 3 weeks after planting, phosphorus contents in corn plants were similar between the control and the treatment with the values of 3.7 and 3.9 mg P/g. In the four weeks, however, phosphorus contents in the control and the treated corn plants were 1.4 and 2.4 mg P/g, respectively. After 7 weeks, phosphorus content in the treated plants was 4 times greater than that in the controL plants (Fig. 3). The result

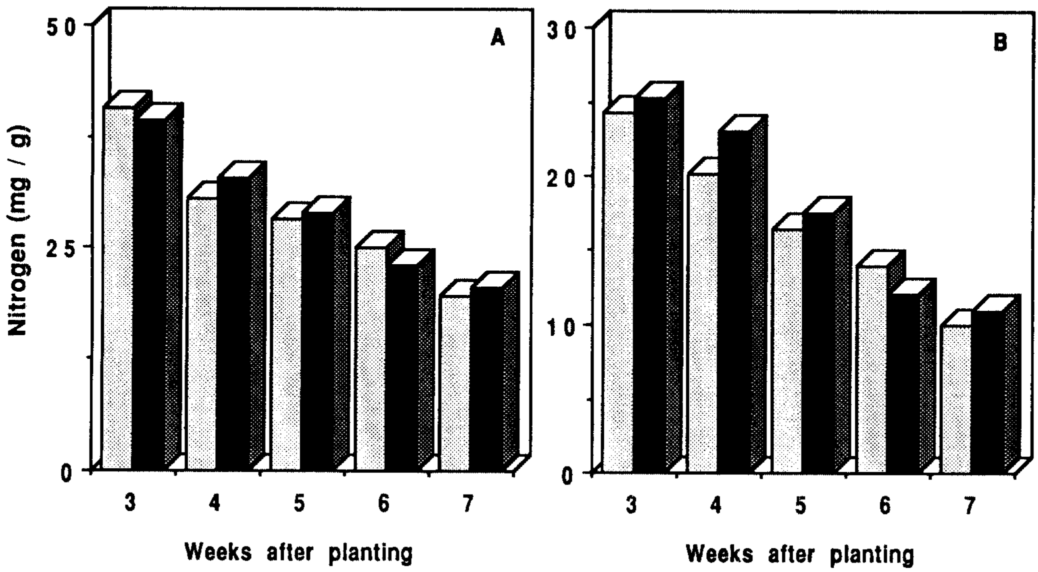

Fig. 2. Nitrogen concentration in the plant tissue of the control (dotted) and treatment (black) in the bell pepper (A) and corn (B).

of the bell pepper was similar with that of the corn. In the 3 weeks after planting, phosphorus contents in the control and the treated bell pepper plants were 4.1 and 4.0 mg P/g, respectively. In the 4 weeks, however, phosphorus content in the treated plants was significantly higher than

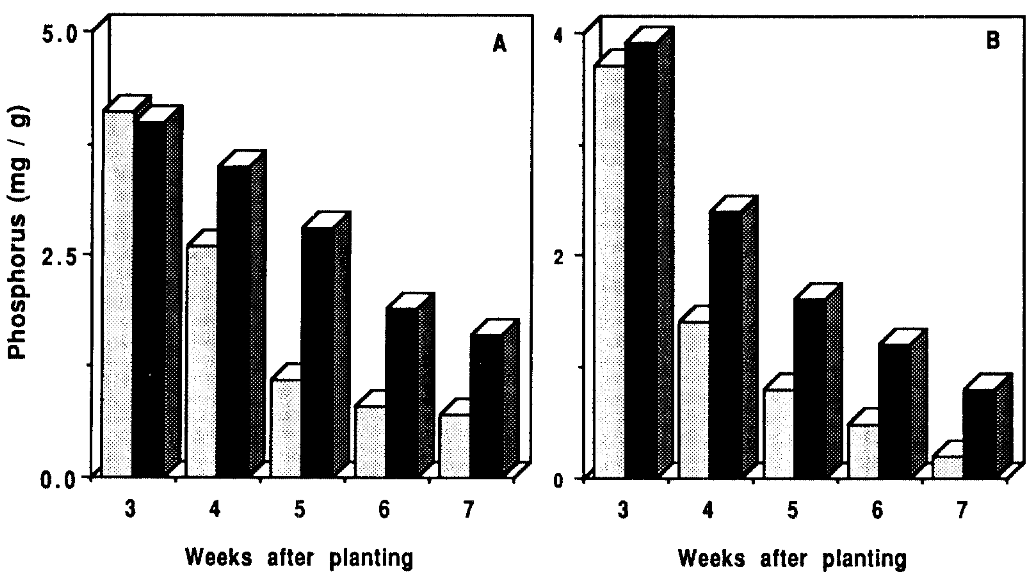

Fig. 3. Phosphorus concentration in the plant tissue of the control (dotted) and treatment (black) in the bell pepper (A) and corn (B).

that in the control plants. After 7 weeks, phosphorus content in the treated plants was above 2 times greater than that in the control plants.

There have been many experiments reported in recent years which have been designed to examine the effects of vesicular-arbuscular mycorrhizal association on plant growth and metabolism (Abbott and Robson, 1984). The results of this experiment prove that the lower growth rates of bell pepper and corn in sterilized soil/sand mixture were due to poor phosphorus nutrition associated with lack of infection by mycorrhizal fungus. Therefore, some means of increasing vesicular-arbuscular mycorrhizal fungus infection is required to increase crop production when growing these crops in soils deficient in these fungi.

摘　　要

균근 형성이 고추와 옥수수의 초기생장에 미치는 효과를 조사하였다. 실험구 식물체의 균근 형성은 발아 후 3주일 후부터 시작되었다. 실험기간 동안에 대조구의 식물들은 균근이 형성되지 않았고 실험구의 식물체 뿌리 조직에서는 많은 균사와 vesicles을 관찰할 수 있었다. 7주일 후 고추와 옥수수 실험구개체들의 초장은 대조구 개체들에 비해 각각 110%와 90% 증가하였고 실험구 개체의 지상부 평균 무게는 고추와 옥수수에서 각각 88%와 71% 증가하였다. 지상부/지하부의 비는 고추와 옥수수가 대조구에서 각각 2.7과 1.8, 그리고 실험구에서 각각 4.3과 2.7이었다. 실험구 식물의 인산함량은 대조구에 비해 높았지만 질소함량은 실험구와 대조구 간에 유의차가 없었다.

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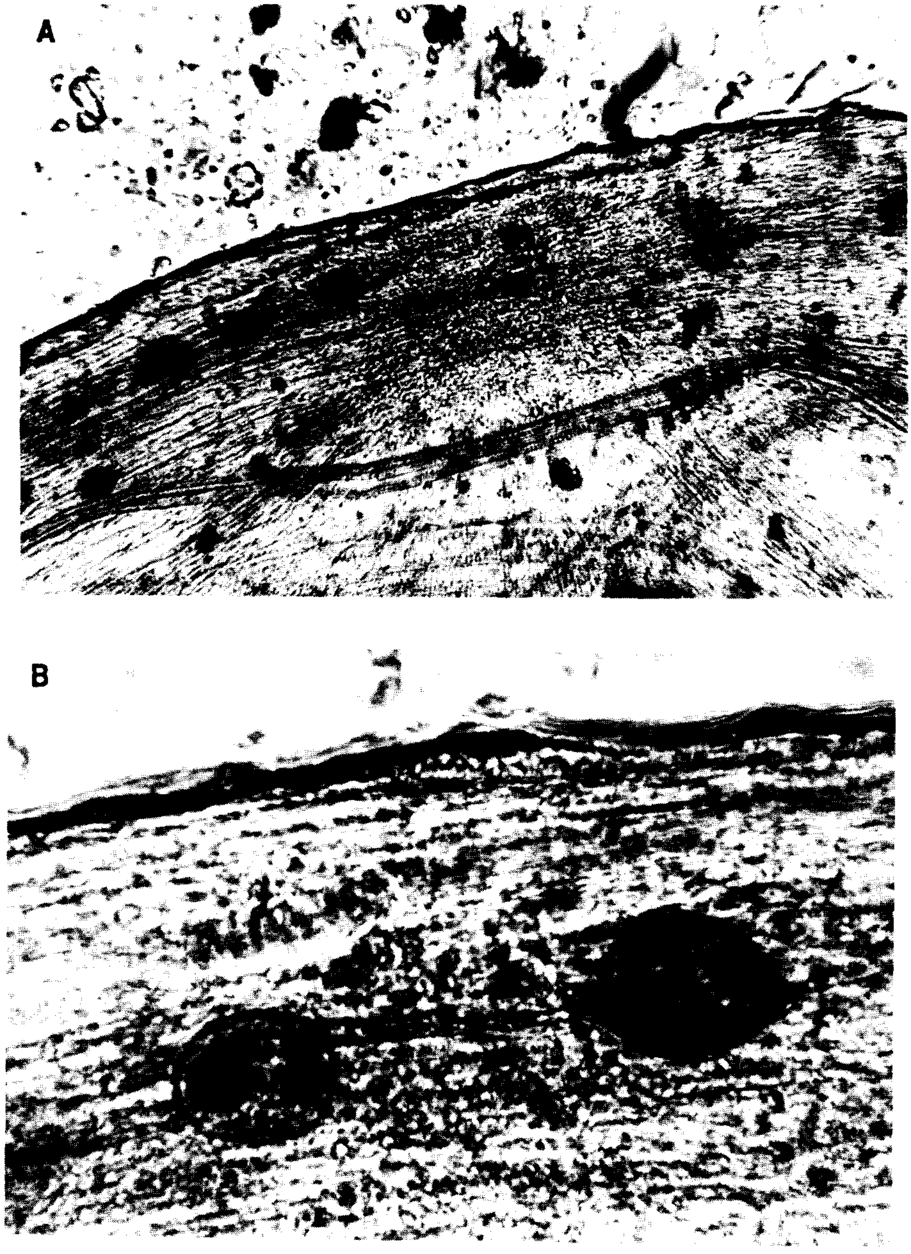

Plate 1. Vesicles and hyphae in root cortex of the bell pepper. A (×100), B (×430).