

# The Influence of Four Species of Vesicular Arbuscular Mycorrhizas on the Growth of Three Legume Plants

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**ABSTRACT.** Inoculation with mycorrhizas resulted in significant increase in all parameters measured in this investigation. The bean, soybean and cowpea plants showed a significant response to all the applied inocula compared with non-mycorrhizal plants. The highest percentage increase in dry matter production was found in cowpeas. This increase was nearly fourfold when inoculated with *Glomus etunicatum*, *G. geosporium* and *G. clarum* and only twofold with *G. macrocarpium*. In beans, the increase in dry matter production was twofold only with all inoculants. While in soybean, the increase was less than twofold. Differences amongst inocula were not significant in bean or soybean plants, but they did show significant differences in the cowpea plants.

## Introduction

Vesicular arbuscular mycorrhizas (VAM) are common in soil and can increase plant nutrient uptake and growth (Al-Garni and Daft<sup>[1]</sup>; Vaast and Zasoski<sup>[2]</sup>). VAM fungi are distinctive for their wide host range, but species differ significantly in their ability to increase growth of host plants (Daniels & Menge<sup>[3]</sup>; Son *et al.*<sup>[4]</sup>). The effectivity of a certain endophyte is affected by many factors such as host plant and mycorrhizal fungus (Menge *et al.*<sup>[5]</sup>; Nemeč,<sup>[6]</sup>). Equal spore numbers of various VAM species are used as inoculum but spore potential may vary, owing to wide differences in spores size, viability or germinability.

The aim of the work reported in this paper was to compare the root colonization, growth enhancement and spore production resulting from inoculation of three legume plants with four species of *Glomus*, isolated from Saudi Arabia and Florida in the U.S.A.

## Materials and Methods

### *Growth Conditions and Treatments*

The host plants used in this study were *Glycine max* (Soybean); *Phaseolus vulgaris* (Bean); and *Vigna sinensis* (Cowpea).

The plants were grown in 5" pots filled with washed and horticulturally sterilized sand (1.1 kg/pot<sup>-1</sup>) mixed with rock phosphate (29% P<sub>2</sub>O<sub>5</sub>) at the rate of 0.2 g/kg<sup>-1</sup> sand. The mycorrhizal treatment were inoculated by pipetting 10 ml of a suspension containing around 250 spores into holes made in the sand of each pot. Control plants of each host received an equivalent amount taken from a suspension free of all active unites. Five replicates were prepared for each treatment. All plants were grown in the greenhouse and had supplementary illumination for 13 hours, and temperature of 18°C at night and 30°C during the day time. Plants were fed twice weekly with 30 ml of the Hoagland's solution minus the phosphate salt.

The comparative effects to the mycorrhizal inocula on plant growth were determined by the following: plant height at approximately 65 days after sowing, total dry weight at harvest (10 weeks after sowing), total and infected root length at harvest a random sample of roots had been cleared and stained following the procedure of Phillips and Haymas<sup>[7]</sup>, and spore numbers of each mycorrhizal fungus using the wet sieving and decanting technique (Gerdemann and Nicolson<sup>[8]</sup>).

### **VAM Fungal in Inocula**

The mycorrhizal spores used in this study were *Glomus etunicatum* (Becker and Gerdemann); *Glomus geosporium* (Walker); *Glomus macrocarpium* (Berch and Fotin) and *Glomus clarum* (Nicolson and Gerdmann). *G. clarum* was collected in Florida (U.S.A.); while the other three species were collected in Saudi Arabia. All spores were multiplied in pot cultures using maize as a good host and equivalent amounts of inoculum from the different species were taken into consideration (about 250 spores/pot<sup>-1</sup>).

### **Statistical Analysis**

The data collected were statistically tested for significance by analysis of variance, using Fisher's LSD test for the determination of the significant differences amongst treatments.

## **Results and Discussion**

### **(i) Effect of Mycorrhizal Inoculation on Plant Growth**

Inoculation with mycorrhizas resulted in significant increase in all parameters measured in the investigations *i.e.* plant height; dry weight and total and infected root length. In the terms of height, the bean, soybean and cowpea plants showed a significant response to all the applied inocula compared with the corresponding non-mycorrhizal plants (Table 1). Dry matter production of the three legumes were also significantly larger than the controls when inoculated with *Glomus etunicatum*, *G. geosporium*, *G. macrocarpium* and *G. clarum* (Table 2). The highest percentage increase in dry matter production was found in cowpeas, beans and soybeans respectively. This increase was nearly five fold for the cowpeas when inoculated with *G. etunicatum*, *G. geosporium* and *G. clarum* and only two fold with *G. macrocarpium*. In the case of beans, the increase in dry matter production was two fold only with all inoculants. While in soybean, the increase was less than twofold.

TABLE 1. Heights (cm/plant<sup>-1</sup>) of three legume plants as influenced by inoculation with *Glomus etunicatum*, *G. geosporium*, *G. macrocarpium* and *G. clarum*.

Inoculum	Legume plants					
	Bean	% increase	Soybean	% increase	Cowpea	% increase
<i>G. etunicatum</i>	44.2 b	51	105.3 c	48	148.3 b	220
<i>G. geosporium</i>	43.6 b	49	108.1 c	52	139.8 b	202
<i>G. macrocarpium</i>	41.3 b	41	93.9 b	32	138.6 b	199
<i>G. clarum</i>	44.5 b	52	103.0 c	45	145.7 b	215
Non-mycorrhizal	29.2 a	0	71.2 a	0	46.3 a	0

\*Within columns, numbers followed with the same letter are not significantly different ( $P < 0.05$ ).

TABLE 2. Total dry weight (g/plant<sup>-1</sup>) of three legume plants as influenced by inoculation with *Glomus etunicatum*, *G. geosporium*, *G. macrocarpium* and *G. clarum*.

Inoculum	Legume plants					
	Bean	% increase	Soybean	% increase	Cowpea	% increase
<i>G. etunicatum</i>	1.76 b	123	2.27 b	71	2.83 c	405
<i>G. geosporium</i>	1.82 b	130	2.06 b	55	2.67 c	377
<i>G. macrocarpium</i>	1.61 b	104	1.88 b	41	1.29 b	130
<i>G. clarum</i>	1.68 b	113	1.87 b	41	2.65 c	373
Non-mycorrhizal	0.79 a	0	1.33 a	0	0.56 a	0

\*Within columns, numbers followed with the same letter are not significantly different ( $P < 0.05$ ).

Differences between inocula were not significant in bean or soybean plants, but they did show significant differences in the cowpeas. The dry matter values in cowpea were produced by *G. etunicatum* (405% increase); *G. geosporium* (377% and *G. clarum* (373%)) respectively. Whereas, the lowest was found in plants inoculated with *G. macrocarpium* (130%). Evidence in increasing that endophytes can differ in their ability to improve plant growth (Mosse<sup>[9],[10]</sup>; Powell,<sup>[11]</sup>; Abbott and Robson,<sup>[12]</sup>; Rangeley *et al.*<sup>[13]</sup>).

### (ii) Development of Mycorrhizal Infection in Plant Roots

The total and infected root lengths (m/plant<sup>-1</sup>) of the three legumes are presented in Table 3. Generally, root growth was increased by the presence of VA-mycorrhizas presumably because of the better plant nutrition. In the beans, *Glomus geosporium* gave a significant greater total root length than inocula *G. etunicatum*, *G. clarum* and the control treatment. All endophytes produced significantly longer root in bean and cowpea. In soybean, *G. clarum*, *G. etunicatum* and *G. macrocarpium* showed significantly longer root systems than the respective controls and the plants inoculated with *G. geosporium*. Comparing the mycorrhizal beans, *G. geosporium* produced the largest root system followed by *G. macrocarpium*, *G. clarum* and finally *G. etunicatum*.

TABLE 3. Total and infected root length (m/plant<sup>-1</sup>) of three legume plants as influenced by inoculation with *Glomus etunicatum*, *G. geosporium*, *G. macrocarpium* and *G. clarum*.

Inoculum	Legume plants					
	Bean		Soybean		Cowpea	
	Total	Infected	Total	Infected	Total	Infected
<i>G. etunicatum</i>	83.3 b	23.1 b	68.2 b	30.3 d	70.8 d	48.1 cd
<i>G. geosporium</i>	103.6 c	33.4 c	57.3 a	26.1 c	64.9 c	45.6 c
<i>G. macrocarpium</i>	98.3 bc	22.3 b	64.1 b	20.6 b	46.3 b	23.7 b
<i>G. clarum</i>	84.9 b	23.5 b	67.9 b	28.5 d	72.3 d	56.3 d
Non-mycorrhizal	43.9 a	0.0 a	44.5 a	0.0 a	17.6 a	0.0 a

\*Within columns, numbers followed with the same letter are not significantly different ( $P < 0.05$ ).

Infected root length also varied significantly amongst endophytes (Table 3). The mycorrhizal root length of bean was greatest with *G. geosporium* than the other inoculated treatments. In soybean, *G. etunicatum* and *G. clarum* gave the larger infected root lengths than that observed by *G. geosporium* and *G. macrocarpium*. Cowpea gave the maximum response with *G. clarum* where the infected root length recorded was 56.3 m with *G. clarum*; 48.1 m with *G. etunicatum*; 45.6 m with *G. geosporium* and only 23.7 m with *G. macrocarpium*.

Results in Table 4 show the number of spores produced in the rhizosphere of the three legumes. The highest spore numbers of all inocula, were produced by cowpea followed by soybean and finally by beans.

TABLE 4. Number of mycorrhizal spores (spore/plant<sup>-1</sup>) extracted from the rhizosphere of three legume plants inoculated with *Glomus etunicatum*, *G. geosporium*, *G. macrocarpium* and *G. clarum*.

Inoculum	Legume plants		
	Bean	Soybean	Cowpea
<i>G. etunicatum</i>	2060 c	3678 b	8567 c
<i>G. geosporium</i>	2516 d	3606 b	6654 b
<i>G. macrocarpium</i>	5900 e	9318 d	10769 d
<i>G. clarum</i>	1260 b	4889 c	18635 e
Non-mycorrhizal	0 a	0 a	0 a

\*Within columns numbers followed with the same letter are not significantly different ( $P < 0.05$ ).

The present study has established that inoculation with different VAM fungi had different effects on the growth of beans, soybeans and cowpeas, the *G. etunicatum* was always the most efficient in stimulating plant growth. *G. macrocarpium* was poorest and *G. clarum* and *G. geosporium* were most consistently good.

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## تأثير أربعة أنواع من فطريا الميكورايزا الحويصلية الشجيرية على نمو ثلاثة نباتا بقولية

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المستخلص . أظهر نتائج الدراسة الحالية جدوى التلقيح بالميكورايزا في زيادة إنتاج المحاصيل الزراعية . فقد أوضحت النتائج إستجابة النباتا المدروسة (فول ، فول الصويا ، اللوبيا ) لجميع أنوا الميكورايزا التي تم اختبارها بالمقارنة بالنباتا غير الملقحة . وقد تبين أن أعلى إنتاجية في الوزن سجلت في نباتا اللوبيا الملقحة بجراثيم فطريا الجلومس إيتانيكاتم والجلومس جيوسبوريم والجلومس كلارم حيث بلغت أربعة أضعاف الوزن الجاف للنباتا غير الملقحة . في حين بلغت الزيادة ضعفين فقط عند التلقيح بجراثيم الجلومس ماكروكاربم

وبالنسبة لنباتا الفول فلم تتعدى الزيادة في الوزن الجاف نتيجة التلقيح بالميكورايزا الضعفين مقارنة بنباتا المعاملة غير الملقحة . أما نبا فول الصويا فقد كانت الزيادة أقل من الضعفين . وفيما يتعلق بالاختلافا بين اللقاحا المستخدمة ، فقد أظهر الدراسة عدم وجود اختلافا جوهرية بينها مع نباتا فول الصويا والفول ، إلا أنها كانت جوهرية مع نبا اللوبيا .