Mycorrhiza (1995) 5:229–231

ORIGINAL PAPER

Ahmad M. Al-Raddad

Mass production of *Glomus mosseae* spores

Abstract Five crops inoculated with *Glomus mosseae* were grown for 10 weeks and the development of mycorrhizal infection and sporulation were assessed. Infected roots from pot cultures of different ages were used to examine the host effect on the development of mycorrhizae. The effectiveness of each host was assessed by measuring spore numbers. For all hosts, the percentage of root length infected increased rapidly up to 10 weeks after sowing. Infectivity of root inocula increased with increasing percentage of root length infected with the inoculum for all crops, except where large numbers of mature spores (1755) had been produced on barley. The highest spore numbers were achieved in the rhizosphere of barley plants, followed by chickpea and beans. The lowest spore numbers were found in the rhizosphere of corn and okra plants. The type of the crop as well as the harvest date greatly influenced the size of the spore population and the extent of root colonization of *G. mosseae*.

Key words *Glomus mosseae* · Sporulation Mycorrhizae · Colonization

Introduction

Vesicular-arbuscular (VA) endophytes are obligate biotrophic fungi forming symbiotic relationships with the roots of many plants (An et al. 1993; Sreeenivasa et al. 1993; Al-Raddad 1987). The broad use of VA mycorrhizal (VAM) fungi has been limited because none of the VAM fungi have been cultivated in vitro (Hepper 1984) and it is, therefore, difficult to obtain large quantities of prime inoculum. Many VAM fungi produce asexual chlamydospores or azygospores, which are thought to be of primary importance in the life cycles of the fungi, although root- and soil-borne vesicles as well as mycelium also contribute to their reproductive potential (Powell 1976). The growth stage and physiology of host plants have been postulated to influence spore production of endomycorrhizal fungi (Simpson and Daft 1990; Hetrick and Bloom 1986; Luedders et al. 1979). The different hosts suggested for inoculum production include cassava (Potty 1985), Bahia grass (Struble and Skipper 1988) and Rhodes grass (Sreenivasa and Bagyaraj 1988). The present study has sought to provide information on the effect of host plants on spore production of a VAM fungus and the relationship of spore production to root infection and plant age.

A. M. Al-Raddad
Department of Plant Protection, Faculty of Agriculture, University of Jordan, Amman, Jordan

Materials and methods

The growth medium was a 3:1 sand:soil mixture containing 1% organic matter and with pH 7.5. Each 15-cm plastic pot was inoculated with the mycorrhizal fungus *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe. The primary inoculum was spores isolated from spinach in the Jordan Valley and produced in pot cultures on chickpea (Al-Raddad 1993); it consisted of a root-soil-spore mixture. Aliquots (100 g) of this inoculum were mixed with the growth medium to give an initial inoculum density of 20 chlamydospores/100 g dry soil. Five crops were used as hosts: barley (*Hordeum vulgare* L.), beans (*Phaseolus vulgaris* L.), chickpea (*Cicer arientinum* L.), corn (*Zea mays* L.) and okra (*Hibiscus esculentus* L.). Four seeds of each crop were planted per pot and seedlings were thinned to two per pot 2 weeks after sowing. Plants were grown in the greenhouse under natural illumination and watered with tap water as needed. For each host, 12 replicate pots were randomly distributed.

Four weeks after sowing, seedlings of four pots were removed and mycorrhizal infection was assessed by a modified clearing and staining technique (Phillips and Hayman 1970), and spore population by the wet-sieving method (Gerdemann and Nicolson 1963). Two further harvests of four random pots per treatment were made at 7 and 10 weeks after planting. A 5-mm horizontal section was cut across each root system and the root segments were cleared by heating for 10 min in a 10% (w/v) potassium hydroxide solution. Cleared samples were stained for 5 min in hot lactophenol containing 0.05% trypan blue. The percentage of the total root length infected by the mycorrhizal fungus was estimated microscopically (Bierman and Linderman 1981). Since the roots were small at the first harvest, in this case the entire root

systems were cleared, stained and examined. After every harvest, a 100-g moist soil sample from each pot was wet sieved. Spores were collected betwen the 50-μm and 300-μm sieves and counted. Spore populations were expressed per 100 g oven dry soil after soil moisture determination.

Results

The type of crop had the greater effect on spore numbers and root colonization, although harvest date also played an important role in spore production (Tables 1, 2). The percentage of root colonization by *G. mosseae* was extremely variable at the early harvest. By the third harvest, the root systems were 16–33% colonized. Spore numbers did not correlate significantly with percentage of root colonization. The highest spore numbers were achieved in the rhizosphere of barley plants, followed by chickpea and beans. The lowest spore numbers were found in the rhizosphere of corn and okra plants. Sporulation occurred at the highest rate by the second harvest on barley, chickpea, beans and okra, while a lower rate was observed between the second and the third harvests. The percentage root infection increased slowly in beans, chickpea and okra, while barley and corn roots showed a higher percentage of VAM infection by the two earlier harvests. The severity of root colonization in all crops except okra was stable around 50% at the second harvest. By the third harvest, bean roots exhibited higher severity and percentage of root infection than the other host roots.

Table 1 Spore numbers per 100 g oven dry soil at three harvests starting 4 weeks after sowing. Means within each column followed by the same letter are not significantly diferent at the 5% level according to Duncan's multiple range test

Host	First harvest	Second harvest	Third harvest
Barley	107	1504 a	1755 a
Beans	22	505 c	808 bc
Chickpea	73	940 b	922 b
Corn	22	185 d	448 d
Okra	89	420 cd	670 c

Table 2 Root colonization by *Glomus mosseae* of different hosts at three harvests. Within-column values with at least one superscript in common are not significantly different at the 5% level. *X1* Percentage infection (incidence), *X2* severity of root colonization on a relative scale of 0 to 10

Host	First harvest		Second harvest		Third harvest	
	X1	X2	X1	X2	X1	X2
Barley	20	2.5	26 a	4.4 a	25 ab	3.5 b
Beans	0	0	4 b	5.5 a	33 a	6.4 a
Chickpea	0	0	8 b	5.0 a	16 bc	3.0 b
Corn	16	4.8	30 a	5.0 a	15 cd	2.0 b
Okra	0	0	6 b	2.3 b	6 d	2.0 b

Discussion

Harvest date was an important factor having a significant effect on spore numbers. Spore population and average root colonization increased with harvest time but crop type had a greater effect on spore numbers than harvest time. The percentage of root system colonized by *G. mosseae* was extremely variable in the early harvests, the spore numbers varying considerably among plants without any significant correlation with infection percentage. This agrees with early findings where no correlation was found between spore number and root infection or between root colonization and plant growth responses (Al-Raddad 1991; Kapulnik and Kushnir 1991).

Spore numbers tended to increase with the size of barley and chickpea plants. Barley had a large root system due to the rapid root growth of the graminaceous crop under the experimental conditions. Monocots with rapidly developing, fibrous root systems are ideal trap plants. High inoculum densities were often associated with high levels of barley root infection early in the growing season. Large plant size has been associated with increased levels of sporulation, probably because larger plants often have more extensive root systems than smaller plants, allowing greater mycorrhizal colonization and sporulation (Saif and Khan 1977). Manjunath and Habte (1991) demonstrated that species differing in growth rates could differ in their mycorrhizal dependency, even when they had similar root morphological characteristics. Root mass can in turn be influenced by available soil or pot volume. The relatively low colonization severity of barley and chickpea roots resulted from the short duration of the experiment, and the dilution of infection was due to extensive spread of the roots exceeding the velocity of fungal distribution.

Newly formed spores may require a resting period and be unable to form infection points promptly; thus spore populations reached a high level by the second harvest in all crops. Mycorrhizal spores germinate randomly in steamed soil, producing a network of mycelium which spreads throughout the soil and gives many potential contact and infection points between the mycelium and the root system (Daniels et al. 1981). Corn plants showed the lowest spore build up in the rhizosphere but the highest percentage of root infection by the second harvest. This phenomenon can be explained by host specificity; corn roots are easily colonized by the hyphae of *G. mosseae* and have shown plant growth responses in other experiments (Simpson and Daft 1990). Beans showed the highest severity of root colonization by the final harvest due to high sporulation and small root volume, while lower final spore populations with the lowest colonization severity occurred with okra and corn roots. The differences observed in this work suggest that more attention should be given to equalizing inoculum potential in future experiments when comparing VAM species for efficiency of infection.

Acknowledgement The author thanks Dr. Ghazi Al-Karaki for his valuable suggestions.

References

Al-Raddad A (1987) Effect of three vesicular arbuscular mycorrhizal isolates on growth of tomato, eggplant and pepper in a field soil. Dirasat 14:161–168

Al-Raddad A (1991) Response of bean, broad bean and chickpea plants to inoculation with *Glomus* species. Sci Hortic 46:195–200

Al-Raddad A (1993) Distribution of different *Glomus* species in rainfed areas in Jordan. Dirasat 20:165–182

An Z, Shen T, Wang H (1993) Mycorrhizal fungi in relation to growth and mineral nutrition of apple seedlings. Sci Hortic 54:275–285

Bierman B, Linderman R (1981) Quantifying vesicular-arbuscular mycorrhizae: proposed method towards standardization. New Phytol 87:63–67

Daniels B, McCool P, Menge J (1981) Comparative inoculum potential of spores of six vesicular-arbuscular mycorrhizal fungi. New Phytol 89:385–391

Gerdemann J, Nicolson T (1963) Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Trans Brit Mycol Soc 46:235–244

Hepper C (1984) Isolation and culture of vesicular arbuscular mycorrhizal (VAM) fungi. In: Powell CL, Bagyaraj DJ (eds) VA mycorrhiza. CRC Press, Boca Raton, Fla, pp 95–112

Hetrick B, Bloom J (1986) The influence of host plant on production and colonization ability of vesicular-arbuscular mycorrhizal spores. Mycologia 78:32–36

Kapulnik Y, Kushnir U (1991) Growth dependency of wild, primitive and modern cultivated wheat lines on vesicular-arbuscular mycorrhizal fungi. Euphytica 56:27–36

Luedders V, Carling D, Brown M (1979) Effect of soybean plant growth on spore production by *Glomus mosseae.* Plant Soil 53:393–397

Manjunath A, Habte M (1991) Relationship between mycorrhizal dependency and rate variables associated with P uptake, utilization and growth. Commun Soil Sci Plant Anal 22:1423–1437

Phillips J, Hayman D (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Potty V (1985) Cassava as an alternate host for multiplication of vesicular-arbuscular mycorrhizal fungi. Plant Soil 88:135–137

Powell C (1976) Development of mycorrhizal infections from *Endogone* spores and infected root segments. Trans Br Mycol Soc 66:439–445

Saif S, Khan A (1977) The effect of vesicular-arbuscular mycorrhizal associations on growth of cereals. 3. Effects on barley growth. Plant Soil 47:17–26

Simpson D, Daft M (1990) Spore production and mycorrhizal development in various tropical crop hosts infected with *Glomus clarum.* Plant Soil 121:171–178

Sreenivasa M, Bagyaraj D (1988) *Cloris gayana* (Rhodes grass), a better host for the mass production of *Glomus fasciculatum* inoculum. Plant Soil 106:289–290

Sreenivasa M, Krishnaraj P, Gangadhara G, Manjunathaiah H (1993) Response of chilli (*Capsicum annum* L.) to the inoculation of an efficient vesicular-arbuscular mycorrhizal fungus. Sci Hortic 53:45–52

Struble J, Skipper H (1988) Vesicular-arbuscular mycorrhizal fungal spore production as influenced by plant species. Plant Soil 109:277–280