

The Abundance of Arbuscular Mycorrhizal Fungi Spores in Rhizospheres of Different Crops

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Abstract: The number of spores of vesicular-arbuscular mycorrhizal (VAM) fungi occurring in the rhizospheric soil of 14 crops was investigated using traditional wet-sieving/decanting methods. The aim of the study was to determine the effect of some agronomy species (Gramineae, Leguminosae, Solanaceae, Labiatae, Cruciferae, Umbellifera, and Alliaceae) on VAM spores numbers in the rhizosphere. The agronomy species were planted under greenhouse conditions in pots containing semiarid soil. The number of spores in the rhizosphere of each species was studied at the middle and end of vegetative plant growth. In the middle of vegetative growth the number of spores increased significantly in the rhizosphere of some species compared to the control (152 spores 10 g^{-1} dry soil). The number of spores was lower in *Raphanus sativa* rhizospheric soil (115 spores 10 g^{-1} dry soil) and higher in *Zea mays* rhizospheric soil (255 spores 10 g^{-1} dry soil). At the end of vegetative growth the number of spores increased only in the rhizosphere of *Triticum aestivum*, *Zea mays*, *Trifolium repens*, *Solanum tuberosum*, *Satureja hortensis*, and *Allium cepa*, and this increase was significant. In this stage of plant growth the number of spores was lowest in *Raphanus sativa* rhizospheric soil (126 spores 10 g^{-1} dry soil) and highest in *A. cepa* rhizospheric soil (453 spores 10 g^{-1} dry soil).

Key Words: Agronomy species, rhizosphere, vesicular-arbuscular mycorrhizal fungi, spore

Farklı Tahl Ürünlerinin Rizosporlarında Arbuskular Mikorizal Fungi Sporlarının Bolluğu

Özet: On dört tahıl ürününün rizosfer toprağında bulunan vesiküler-arbuskular mikorizal fungi sporlarının sayısı geleneksel wet sieving/deconting yöntemleri kullanılarak araştırılmıştır. Bu çalışmanın amacı bazı tarım ürünlerini (Gramineae, Leguminosae, Solanaceae, Labiatae, Cruciferae, Umbelliferae, Alliaceae) rizosferdeki VAM spor sayıları üzerine etkisini belirtmektir. Tarımsal türler sera koşullarında yarı kurak toprağa saksılarla ekilmiştir. Her türün rizosferdeki spor sayısı kontrolle karşılaştırıldığında belirgin bir şekilde artmıştır (her 10 g^{-1} kuru toprakta 152 spor). Bu oran *Raphanus sativa* rizosfer toprağında daha düşük (her 10 g^{-1} kuru toprakta 115 spor), *Zea mays* ta ise daha yüksektir (her 10 g^{-1} kuru toprakta 255 spor). Vejetatif gelişimin sonunda spor sayıları artmış fakat sadece *Triticum aestivum*, *Zea mays*, *Trifolium repens*, *Solanum tuberosum*, *Satureja hortensis*, *Allium cepa* 'da bu artış belirgindir. Bitki gelişiminin bu aşamasında, *Raphanus sativa* rizosferik toprağında en düşük (Her 10 g^{-1} kuru toprakta 126 spor), *A. cepa* rizosferik toprağında en yüksektir Her 10 g^{-1} kuru toprakta 453 spor).

Anahtar Sözcükler: Tahıl ürünleri, rizosfer, vesiküler-arbuskular mikorizal fungi, spor

Introduction

Plants of most taxa are mycorrhizal with fungi of the order Glomales (1), and soils supporting naturally occurring or cultivated hosts harbor these fungi (2,3). Knowledge of the relationships between mycorrhizal fungi and their hosts in natural habitats is limited. Mycorrhizal effects on plants growing in non-sterilized soil must be considered from the standpoint of the mycorrhizal fungal community, not just of individuals

from the community, for pure cultures of Glomales fungi do not occur in nature. In agricultural and unmanaged habitats, over a dozen species are usually found (4-6).

Spore quantification has been very useful for evaluating the level and diversity of mycorrhizae because spores are highly resistant to adverse conditions (7) and may reflect the previous history of a mycorrhizal symbiosis in the soil (3). Changes in spore production may be due to drought (8). In lowland humid tropics,

spore abundance varies by season, with highest abundance occurring in the dry season (9,10), and is related to low nutrient availability and plant phenology, among others factors.

Given the great importance of these endophytes in natural systems, it is important to identify and to study the distribution and abundance of mycorrhizal fungi. The aim of the present study was to quantify vesicular-arbuscular mycorrhizal (VAM) fungi spore abundance and richness in the rhizosphere of 14 different crops and vegetables. The results of this study may be helpful in the programming of plant rotation.

Materials and Methods

Materials and Experimental Design

A surface-soil sample (0-15 cm) was obtained from a field in Hamadan, Iran, and 3 kg of the soil was put in 20-cm diameter pots, which were arranged in a completely randomized experimental design with 3 replicates.

The study included 7 plant families, including Gramineae (*Triticum aestivum* and *Zea mays*), Leguminosae (*Trifolium repens* and *Cicer arietinum*), Solanaceae (*Solanum tuberosum* and *Solanum lycopersicum*), Labiatae (*Ocimum basilicu* and *Satureja hortensis*), Cruciferae (*Lpidium sativum* and *Raphanus sativa* var. *radicola*), Umbelliferae (*Petroselinum hortense* and *Anetum graveolens*), and Alliaceae (*Allium cepa* and *Allium sativum*). All were planted in the same soil in a greenhouse; 6 pots of each species were planted and 6 plant-free pots were prepared as controls.

Soil sampling

Six randomly selected plants of each species in each block were carefully dug out from their pots in the middle and end of vegetative growth. The soil strongly adhering to roots was considered rhizospheric soil.

Soil physical and chemical analyses

Air-dried soil was subsequently crushed and sieved to pass through a 2-mm mesh screen for particle-size analysis using the hydrometer method (11). Calcium carbonate equivalents (CCEs) were measured by the back titration procedure (12). Soil pH and electrical conductivity (EC) were measured in a 1:2 soil/water extract after shaking for 30 min (13). Organic carbon (OC) was analyzed by dichromate oxidation and titration

with ferrous ammonium sulfate (14). Total nitrogen in all samples was determined by the Kjeldahl method (15). Cation-exchange capacity (CEC) and available K were measured according to Bower et al. (16). Available phosphorus was extracted with 0.5 M NaHCO₃ (pH 8.5) and determined spectrophotometrically as blue molybdate-phosphate complexes under partial reduction with ascorbic acid (17).

Microbiological and biochemical analyses

Fresh soil samples were stored at 4 °C for microbiological analysis. VAM fungi spores were isolated from 50-cm³ sub-samples by wet sieving (18) and sucrose gradient centrifugation (19), and were then counted (20). Basal respiration was measured as CO₂ evolved in 5 days (21). Substrate-induced respiration (22) was determined in 72 h. Heterotrophic bacterial and Azotobacter populations were estimated by the plate count method. Soil suspension and dilutions were prepared. Media of soil extract agar (SEA) (23), rose bengal-starch-casein-nitrate agar (RBSCNA), and modified potato dextrose agar (MPDA) were prepared in the lab and used for determination of total soil bacterial, actinomycetes, and fungi populations, respectively (21). Two media were prepared in the lab for study of the Azotobacter population in the soil samples. The first one was Ashby's mannitol agar (24). For inhibition of the growth of gram-positive bacteria and actinomycetes, it was modified by the addition of 1 ml of crystal violet solution (5 g l⁻¹ in ethanol). The second medium for Azotobacter enumeration was LG medium (21). Colony forming units (CFUs) on the solid media were counted after 1 week of incubation at 27 °C (21,24). Soil acid and alkaline phosphatases were analyzed as described by Eivazi and Tabatabai (25). Soil cellulase activity was assayed by the improved method reported by Schinner and Von Mersi (26). The materials and substrates used in the culture media and enzymes assessments were obtained from Merck.

Statistical analyses

Data were statistically analyzed for standard deviation, means were calculated, and Duncan's new multiple range tests were performed to assess the effect of some agronomy species (Gramineae, Leguminosae, Solanaceae, Labiatae, Cruciferae, Umbellifera, and Alliaceae) on VAM spore numbers in the rhizosphere.

Results

The texture of the soil was moderate and determined to be loam (Table 1). The equivalent calcium carbonate (CCE) content of the soil was considerably high ($> 10\%$); thus, the soil was calcareous. Soil pH was > 7 . The 2:1 extract of soil exhibited low EC ($< 0.3 \text{ dS m}^{-1}$). In this semiarid area the mean soil total nitrogen (TN) and organic carbon content were relatively high, 0.11% and 1.02%, respectively. The soil available P determined by the Olsen method was greater (39 mg kg^{-1}) than the critical level (10 mg kg^{-1}). Soil available K was also relatively high (394 mg kg^{-1}). Soil microbial and biochemical activity were also higher compared to other soils of this semiarid region.

In the middle of the plants' vegetative growth the number of spores in the rhizosphere of some plants was higher than that in the control (Table 2). It was relatively high in the rhizosphere of *Z. mays*, *C. arietinum*, *S. lycopersicum*, *A. cepa*, *A. sativum*, *O. bsilicum*, *S. hotensis*, *A. graveolens*, *P. hotanse*, and *L. sativum*; however, in the rhizosphere of *T. aestium*, *T. repens*,

S. tuberosum, and *R. sativa*, it was relatively low (< 152 spores 10 g^{-1} dry soil). The highest number of spores was counted in the rhizosphere of *Z. mays* (255 spores 10 g^{-1} dry soil), which was significantly higher than that in the rhizosphere of *T. repens*, *S. tuberosum*, and *R. sativa*.

At the end of vegetative growth the number of spores increased; however, this increase was significant only in the rhizosphere of *T. aestium*, *Z. mays*, *T. repens*, *S. tuberosum*, *S. hortensis*, and *A. cepa* (Figure).

Statistical analyses showed significant differences between some species at the end of vegetative growth. The number of VAM spores was significantly high in the rhizosphere of *Z. mays*, *T. repens*, *C. arietinum*, *S. tuberosum*, *S. lycopersicum*, *A. cepa*, *A. sativum*, *O. bsilicum*, *A. graveolens*, *P. hotanse*, and *L. sativum*, whereas it was relatively low in the rhizosphere of *T. aestium*, *S. hotensis*, and *R. sativa*. Spore count was the lowest in *R. sativa* rhizospheric soil (126 spores 10 g^{-1} dry soil) and the highest in *A. cepa* rhizospheric soil (453 spores 10 g^{-1} dry soil).

Table 1. Some characteristics of the soil used in this study.

	Texture	Equivalent CaCO ₃	pH	CEC	EC	OC	TN	available P	available K
		%		Cmolc kg ⁻¹	dS m ⁻¹	%	%	mg kg ⁻¹	mg kg ⁻¹
	Loam	10.5	7.9	26.3	0.3	1.02	0.11	39.4	394
Cellulose act.	Alkaline phosphatase act.	Acid phosphatase act.	Azotobacters	Bacteria	Actinomycetes	Fungi	AM spores	SIR	Basal respiration
μg μmol glucose g ⁻¹ soil 24 h ⁻¹	μmol P.N.P g ⁻¹ soil h ⁻¹	μmol P.N.P g ⁻¹ soil h ⁻¹	N *10 ⁴ /g ⁻¹	N *10 ⁷ /g ⁻¹	N *10 ⁴ /g ⁻¹	N *10 ³ /g ⁻¹	N/10 g	mg CO ₂ kg ⁻¹ Soil ⁻¹ day ⁻¹	mg CO ₂ kg ⁻¹ Soil ⁻¹ day ⁻¹
1.43	7.5	2.2	25	1.5	70	1.5	120	572	20

Table 2. Mean number of spores in 10 g of dry rhizospheric soil for each species at the middle and end of vegetative growth.^a

	Control	<i>T. aestium</i>	<i>Z. mays</i>	<i>T. repens</i>	<i>C. arietinum</i>	<i>S. tuberosum</i>	<i>S. lycopersicum</i>	<i>A. cepa</i>	<i>A. sativum</i>	<i>O. bsilicum</i>	<i>S. hotensis</i>	<i>A. graveolens</i>	<i>P. hotanse</i>	<i>L. sativum</i>	<i>R. sativa</i>
Middle of growth	152 abc	145 bc	255 A	130 bc	176 abc	139 bc	218 abc	238 ab	195 abc	150 abc	159 abc	184 abc	170 abc	175 abc	119 c
End of growth	205 g	172 hg	285 Def	282 Fe	389 abc	260 ef	326 cde	453 a	331 bcde	386 abc	240 fg	413 ab	337 Bcde	367 bcd	126 h

^a Means within rows followed by the same letter are not significantly different ($P < 0.01$).

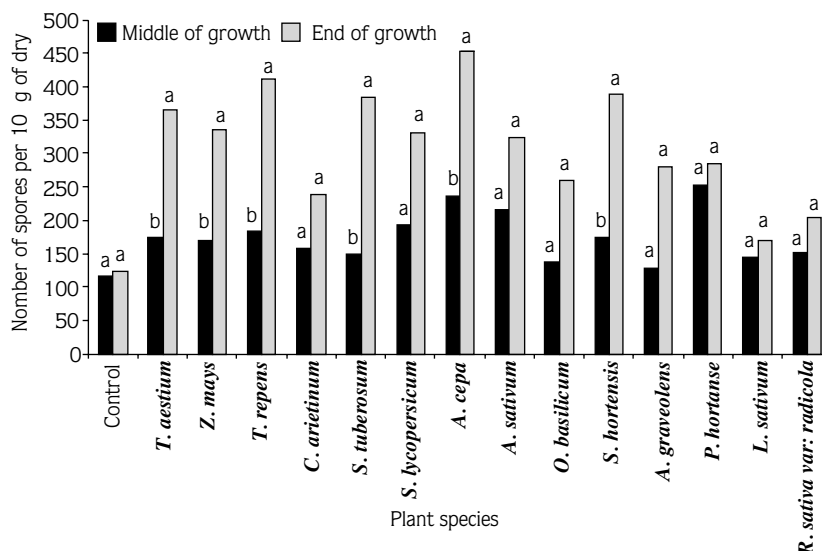


Figure. Number of VAM spores in rhizospheric soil at the middle and end of vegetative plant growth.

Discussion and Conclusion

Crop rotation is an essential component of low-input, sustainable cropping systems. Hayman et al. reported that prior cropping of soil with a non-mycorrhizal plant, such as swede, inhibited mycorrhizal development in seedlings of subsequently planted onion, which is mycorrhizal (27). Similar results have been reported by Hirrel et al., and Harinikumar and Bagyaraj (28,29). Smith found that spore abundance remained low under continuous wheat, but recovered under rotation with pasture (30). Our results are similar to those of the latter study, in which the number of spores in soil decreased significantly by planting *T. aestivum*, *S. hortensis*, and *R.*

sativa cultures, and increased significantly by planting *Z. mays* and *A. cepa*. Therefore, the influence of plant rotation on arbuscular mycorrhizae is significant and should be considered in field management.

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