Journal of
Plant Pathology & Microbiology
ISSN: 2157-7471

Ali et al., J Plant Pathol Microb 2015, 6:1
DOI: 10.4172/2157-7471.1000248

Research Article Open Access

Periodic Existence of Mycorrhizal Fungi in Roots and Non-root Dissident Portions of Some Bulbous Plants

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Abstract

Decaying and senescing scale-like leaves and roots were collected regularly from Botanical Garden, University of the Punjab, Lahore for a period of four months, with an interval of fifteen days. Roots and other portions of both the plants when processed and examined revealed the occurrence of AM fungal structures. However, AM structures were totally missing in *Allium cepa*, and roots of *Amaryllis vittata*. Thick hyphal mats with clusters and clumps of spores were often seen in the decaying scale-leaves. The vesicles in these portions were large sized and thick walled. As regard the seasonal variations, the side by side of hyphal, arbuscular and vesicular infections varied among the samples collected throughout the season. Seasonal variations in Glomalean spore dynamics were observed with respect to number while glomeromycetous species richness varied in the rhizosphere soil of the three plants. The recent research was conducted to evaluate the configuration of occurrence of arbuscular mycorrhizal fungal structures in decaying scale like leaves and root system of three bulbous plants i.e., *Allium cepa*, *Amaryllis vittata* and *Zaphyranthes citrina*.

Keywords: Arbuscular mycorrhizae; Scale-leaves; *Allium cepa; Amaryllis vittata; Zaphyranthes citrine*; Species richness

Introduction

Arbuscular Mycorrhizae (AM) is mutually beneficial relationship amongst fungi and roots of the higher plants. Colonization of roots by mycorrhiza has been revealed to recover development and yield of numerous field crops including leguminous crops, cereals, vegetables and oil crops [1-5]. Mycorrhizal associations increase plant growth and productivity by increasing nutrient element uptake [6] and improving resistance to abiotic [7,8] and biotic [9] stress factors. Plants often benefit from the presence of mycorrhizal associates via a variety of mechanisms including improvement of soil structure, mobilization of essential minerals, enhancement of desiccation resistance, and protection from pathogens and herbivores [10]. Recently some workers have reported that AM also increase the crop tolerance against allelopathy and enhance crop growth under this stress [11,12].

Vesicular arbuscular mycorrhizae are of universal occurrence in roots of 95% of land plants [13,14]. However, during past few decades reports are available about the presence of AM in plant portions other than roots [15,16]. Since than a number of examples have been added to the literature with an emphasis on a much wider occurrence of these associations than reported ever before.

We had planned this study to further add to the information about AM proliferating in roots and non-root portions of some bulbous plants.

Materials and Methods

Sampling of root and non-root portions like scale leaves, dried decaying sheathing leaves on the bulbs of two test bulbous plants viz. *Allium cepa*, *Amaryllis vittata* and *Zaphyranthes citrina*. The sampling was done regularly from February to May. Specific sites (Sites name) were selected for sampling of plants. The root/bulb samples were dug up along with rhizospheric soil. Extreme care was taken to avoid the disturbance of root systems, adhering decayed and semi-decayed scale leaves and epidermis. The samples were brought back to the Lab. in polythene bags. Decaying scale like leaves and fine roots were gently peeled off with the help of forceps, while rest of the bulbous portion along with root system were dipped in a bucket of water for half an hour. The adhering soil was removed with the help of camel hairbrush while washing gently under the tap water. The root system of all samples, scales and other portions were cut up into 1 cm^2 pieces and then fixed in F.A.A. (Formaline: Acetic Acid : Alcohol in 5:5:90 ratio by volume) in properly labeled MacCartney bottles separately. The samples were cleared and stained for analysis of colonization of AM fungi using Phillips and Hayman procedure [15]. The roots were cleared in 10% KOH solution in an autoclave, placed in 0.1N HCl for 2-3 minutes for neutralization and then stained with trypan blue solution (0.05% in lactophenole glycerine). The sample pieces were mounted on the glass slides in a drop of lactic acid and were observed under low power (10x) of the light microscope. Extent of AM infection was recorded with the help of an already calibrated ocular micrometer. This was done by randomly focusing the plant material under the microscope and by measuring the hyphae. The number of vesicles and other structures were also recorded in the same way. Microphotography was done with the help of Minolta X 700 camera with the adapter tube.

Soil adhering to the roots and other portions like bulbs were utilized for screening the associated AM spores. Spore extraction was done by following wet sieving decanting method of Nicolson and Gerdemann [11,12]. Density and diversity of spores was recorded. Spores were identified using synoptic key by Morton [16] and Schenck and Perez [17]. All the data was statistically analyzed by computing Standard

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Received January 19, 2015; **Accepted** January 25, 2015; **Published** January 30, 2015

Citation: Ali M, Adnan M, Azam M (2015) Periodic Existence of Mycorrhizal Fungi in Roots and Non-root Dissident Portions of Some Bulbous Plants. J Plant Pathol Microb 6: 248. doi:10.4172/2157-7471.1000248

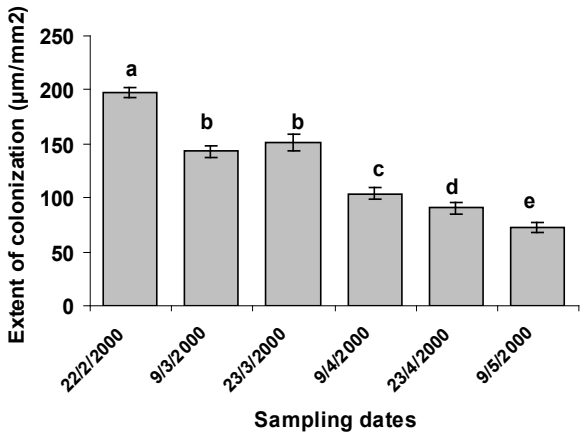

Figure 1: Seasonal changes in extent of AM colonization in root system of *Amayrllis vittata*. Line on data bars show SE of the mean while data bars with different letters show significant difference (p=0.05) as determined by DMRT.

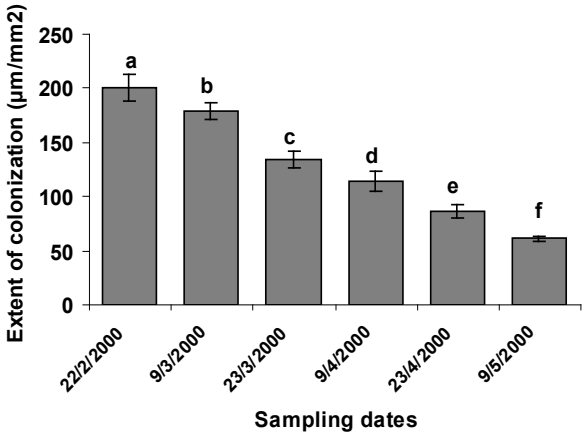

Figure 2: Seasonal changes in status of AM infection in root system of *Zaphyranthes citrina.* Line on data bars show SE of the mean while data bars with different letters show significant difference (p=0.05) as determined by DMRT.

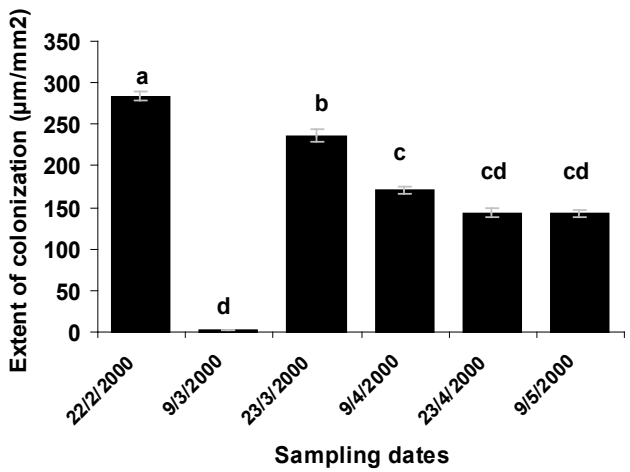

Figure 3: Seasonal changes in status of AM infection in decaying scale leaves of *Zaphyranthes citrina*. Line on data bars show SE of the mean while data bars with different letters show significant difference (p=0.05) as determined by DMRT.

Error, Least Significant Difference; (LSD) and Duncan's New Multiple Range Test (Steel and Torrie [18].

Result and Discussion

AM colonization was completely absent in scales and roots of *Allium cepa*, and roots of *Amaryllis vittata* (members of Amaryllidaceae) may be attributed to existence of immunity to attack by pathogenic fungi. In bulbs of *Allium cepa* resistance to attack by fungus is due to the presence of catecol and protocatechuic acid in the dry, pigmented, outer scale leaves. Members of Amaryllidaceae also produce fungitoxin (Methylene-buty rolactose) in their bulbs, which also diffuses to plant roots (Figures 1-3) and (Tables 1-3).

Sample No	Sampling date	No. of vesicles per mm²	No. of Intramatrical spores per mm²	No. of cells filled with DSEF per mm²
1.	22/2/2000	000.77d ± 0.29	002.44e ± 0.94	008.66e ± 0.66
2.	9/3/2000	002.66b ± 0.577	003.66d ± 1.33	007.33f ± 2.33
3.	23/3/2000	002.66b ± 1.00	005.33c ± 0.19	013.66d ± 1.76
4.	9/4/2000	002.44c ± 0.1	007.99b ± 0.51	015.66c ± 0.66
5.	23/4/2000	002.88a ± 0.39	012.66a ± 3.38	016.00b ± 0.57
6.	9/5/2000	002.44c ± 1.25	012.10a ± 2.27	018.00a ± 4.04
L.S.D		1.36	3.40	3.93

Table 1: Status of various AM structures in decaying scale like leaves of *Amaryllis vittata* at different sampling time.

Sample No	Sampling month	No. of vesicles per mm²	No. of Intramatrical spores per mm²	No. of cells filled with DSEF per mm²
1.	22/2/2000	011.00a ± 0.57	003.66c ± 1.76	--
2.	9/3/2000	010.00b ± 1.52	003.66c ± 0.33	--
3.	23/3/2000	010.33b ± 1.45	003.66c ± 0.33	001.33c ± 0.33
4.	9/4/2000	008.88c ± 3.39	003.89d ± 0.39	006.00b ± 3.00
5.	23/4/2000	006.33d ± 0.03	005.33b ± 0.33	--
6.	9/5/2000	005.33e ± 0.57	006.33a ± 0.36	007.33a ± 0.88
L.S.D		1.77	1.48	2.42

Table 2: Status of various AM structures in root systems of *Zaphyranthes citrina* at different sampling times.

Sample No	Sampling date	No. of vesicles per mm²	No. of Intramatrical spores per mm²	No. of cells filled with DSEF per mm²
1.	22/2/2000	032.55a ± 1.25	004.00e ± 0.57	008.66a ± 2.40
2.	9/3/2000	030.77b ± 0.83	005.33d ± 0.66	004.66c ± 0.33
3.	23/3/2000	026.21c ± 0.29	011.33e ± 1.45	005.33b ± 1.53
4.	9/4/2000	023.88d ± 1.68	022.00d ± 1.52	004.00c ± 1.73
5.	23/4/2000	018.32e ± 1.33	030.33ab ± 1.85	003.66d ± 0.88
6.	9/5/2000	015.55f ± 1.56	032.00a ± 0.57	003.33d ± 1.20
L.S.D		2.35	2.30	2.76

Table 3: Status of various AM structures in scale leaves of *Zaphyranthes citrina* at different sampling times.

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