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INDIGENOUS MYCORRHIZAL SPORE ISOLATION AND IDENTIFICATION FROM WHEAT FIELD, BENGHAZI DISTRICT LIBYA

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are beneficial microorgansims associated with around eighty-percent of land plants. They belong to *Glomeromycota* phylum. In this mutualistic symbiosis, the fungus receives photosynthetic carbon fixed from its host. In return, the host plant gains a plethora of benefits from the fungus such as enhanced nutrient uptakes, and protection against both biotic (soil-borne root pathogens, insect attack); and abiotic (drought, heavy metal pollution, and soil salinity) stresses. The current study was performed to determine the presence of arbuscular mycorrhizal fungal (AMF) spores in a field cultivated with wheat crops (*Triticum aestivum*, L.) during the vegetative stage, and also to investigate soil physiochemical properties effect on AM spores. Three genera of AMF spores were identified in the current study. These genera were *Gigaspora* spp., *Acualospora* spp., and *Glomus* spp. Results showed that *Glomus* spp. was the predominate genus compared to the other two genera.

Keywords: Arbuscular mycorrhizae, glomeromycota, mutualistic symbiosis, and *Triticum aestivum*.

1. INTRODUCTION

As the world populations expected to grow to approximately nine billions by the end of this century, thus, global demands for water and food will increase drastically (Smil, 2000). Unfortunately, the expansion of cultivated area for crop production is restricted and limited; in addition to that, cultivated areas are decreasing due to human activities, and natural degradation. To solve this incumbent dilemma, crop productions must be increased substantially in order to face such enormous demands. Of importance, various plant pathogens such as fungi, bacteria, nematodes, and viruses are responsible for crop production loss. To control these biotic factors, chemical pesticides are applied at horrific scale. Pesticide applications, however, pose serious

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risks on human health and also threaten the fragile ecosystem equilibrium (Igbedioh, 1991; and Jeyaratnam, 1985).

Wheat is one of the most vital cereal and staple crops in the world, and domestication of wheat led to the development of agriculture-based human societies. Wheat is an indispensable crop in the Libyan diet, for instance, its flour used for making bread, pasta and its straw used for feeding livestock. Wheat is classified in the genus *Triticum* belongs to the botanical Family Gramineae; the number of species in the genus varies based on the classification system, but modern classification places the number of species at about 30 (Goncharov, 2011). Wheat production in Libya valued 104000 tonnes in 2008, and will be projected to 165712 tonnes in 2016 (FAO). Low wheat productions reflect the effect of edaphic factors such as soil salinization and low soil fertility; and environmental factors (e.g., wind erosion), thus, most of the country's demand for wheat is imported from Europe.

Arbuscular mycorrhizal fungi (AMF) are omnipresent soil inhabitants that are able to form symbiotic associations with root systems of most plant species. Taxonomically speaking, Schüβler et al (2001) allocated AMF from former Phylum Zygomycota to a newly described Phylum *Glomeromycota* based on small subunit rRNA gene sequences. Colonization of plant roots with AMF improves the host acquisition status of inaccessible minerals (e.g., phosphorus) that otherwise unavailable. Fungal hyphae extend several centimeters, and explore the soil particles efficiently to reach P that beyond root hairs range and translocate it to the host for consumption (Smith and Read, 2008). It has been well documented that mycorrhization protects plant host from important fungal soil-borne pathogens such as *Verticilum*, *Fusarium*, and *Phytophathora* (Aguilar, and Barea, 1997) and pests alike through up-regulations of gene expressions such as jasmonic acid (JA) and salicylic acid (SA) that are quintessential in activating Systematic Acquired Resistance (SAR) (Gutjahr and Paszkowski, 2009; Fu, and Dong, 2013).

The specific objectives of the present study were 1) to isolate and to identify indigenous AMF spores from rhizospheric soils planted with wheat; 2) to maintain start inocula from these isolated AMF spores for future applications; and 3) to raise public and private agricultural sectors awareness for integrating AMF as potentially biocontol agents for controlling plant pathogens and sustainable agriculture production.

2. MATERIAL AND METHODS

2.1 Study site description:

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The present survey was conducted in Benghazi's coastal plain, January 2019. The site of study is located (31.850265, 20.242023). The study area of interest is a cultivated wheat field managed by The Great Man-Made River Project. The climate is relatively semi-arid with an unprecedented annual precipitation exceeding 300 mm. The mean annual temperature in the region was 28 C^{o}. The field is well irrigated, and received sufficient amount of fertilizers: both macronutrients (N, P, K 50:0: 50) and micronutrient (YaraVita Pholate Burgundy, the UK).

Figure 1: Geographic Study location on Map

2.2 Rhizoshpheric soil sampling:

Rhizsphereic soil with wheat roots was collected using a soil auger from the 10-15 cm soil depth. Soil samples were air dried at room temperature for 24 hours. The samples then were grounded and passed through a 2-mm mesh sieve. Soil textural classes were classified according to the United States Department of Agriculture (USDA) triangle system (Soil Survey Staff, 1951), and soil chemical properties such as organic matter (OM), pH, and available phosphorus contents were determined by standard laboratory protocol. All soil analyses were performed at the laboratory of The Great Man-Made River Project, Benghazi, Libya.

2.3 Mycorrhizal spore extraction and identification:

Spores of AMF were extracted following wet-sieving and decanting method (Gerdemann and Nicolson, 1963). One hundred gram of rhizoshpereic soil was suspended in 500 mL of deionized water, and mixed thoroughly for one minute with a glass rod to separate AM spores from soil particles. The soil mixture was left for one hour, and decanted through series of sieves 500, 250, 45 µm. Only the content retained in 45µm sieve was washed with deionized water and poured into Perti dish. Under the dissecting microscope, spores were picked via a micropipette glass and transferred to microscopic slide. The spores then were examined under a compound microscope at 400x magnification (Olympus microscope camera, Japan). Spore morphological characteristics such as spore wall structure, and spore color were utilized for identification. Helpful online website, viz. INVAM website (http://www. invam.caf.wvu.edu), was used for further identification and verification.

3. RESULT AND DISCUSSION

3.1 Soil physio-chemical characterizations:

The data presented in Table1 showed that soil texture was silty-clay (35%, and 43% respectively). As shown also in Table 1., total nitrogen and phosphorus contents were significantly low compared to potassium. The soil pH is generally alkaline as shown in Table 1. Soil pH dramatically affects both AMF spore density and germination. The optimum pH for the highest AMF spore numbers is between (6 to 8) (Isobe et al., 2007), however, some exceptions can be found depending on the specific AMF strains. Mycorrhizal spore population in our study was low (data not shown) and that in agreement with Bhardwaj et al (1997) who reported that sandy soil favors high AMF spore population. Furthermore, AMF spore numbers tend to be more abundant in the summer season than in the spring season, as was the case of our study (Hayman, 1970; Sturmer and Bellei, 1994).

Table 1: Soil physical and chemical proprieties

Parameter	Value
Soil texture	Silty clay
Sand (%)	22
Silt (%)	43
Clay (%)	35
WHC (%)	35.52
HC (cm h^{-1})	2.40
EC (dS/m)	0.5
CEC (Cmole kg^{-1})	25
pH	8.2
OM (%)	1.24
N (%)	0.1
P (ppm)	11
K (ppm)	995

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Note: WHC, water holding capacity; HC, hydraulic conductivity; EC, electrical conductivity; CEC, cation exchange capacity; OM, organic matter; N, total nitrogen; P, available phosphorus; K, available potassium.

3.2 AMF spores isolation from the study site.

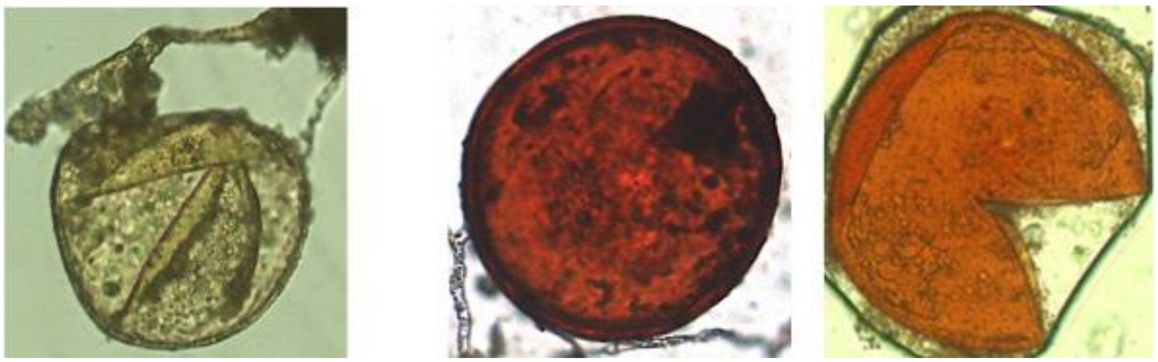

Figure 2: Microscopic photos of AMF spores collected from cultivated wheat field under light compound microscope (40^x). *Acualospora* spp. (a), *Gigaspora* spp. (b), and *Glomus* spp. (c).

Spores of three AMF genera were determined as *Gigaspora* spp., *Acualospora* spp., and *Glomus* spp. belonging to three AMF families' *viz*., *Gigasporaceae*, *Acaulosporaceae*, and *Glomeraceae*, respectively. The diversity of AMF spores in the present study was low in comparison with other studies conducted on wheat (Bhardwaj *et al*. 1997; Abdelhalim *et al*. 2013; and Yaseen *et al*., 2017) and that could be attributed to several factors such as intensive tillage, and lack of crop rotation practices. In addition, Castillo *et al*., 2006 found that conventional tillage significantly reduced the number of AMF propagules in acidic soils. In agreement with our results, Uhlmann *et al*. reported *Glomu*s spp. was the most predominate AMF strains under arid environment.

4. CONCLUSION

Arbuscular mycorrhizae are obligate symbiotic fungi that associate with roots of many vascular plants, except few plant families such as Brassicaceae, Caryophyllace, and Chenopodiaceae that are non-mycorrhizae (Smith and Read, 2008). Three genera of AMF spores in the current study were reported as *Gigaspora* spp., *Acualospora* spp., and *Glomus* spp. It is unclear whether soil *physio-chemical* properties or agricultural practices (*i.e.*, tillage and lack of crop rotation) had a neutral, negative, or positive influence on AMF spore compositions, and diversity in the surveyed field. Therefore, further research is clearly needed to elucidate the exact role that each factor plays independently on AMF spores abundance. To the best of authors' knowledge, our study was the first report in isolating and identifying indigenous AMF spores from Benghazi District soils. Spores isolated here will be inoculated on mycorrhizal host, e.g., tomato or onion, for producing more AMF propagules to serve as a start inoculum for future research. Moreover, commercial AMF incoula productions will begin at a small scale to be used as paradigm on plant productivity, and yields. This will stimulate much attention from people in agricultural sectors on myocrrhizal benefits.

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