Diversity and mycorrhizal potential of arbuscular mycorrhizal fungi in two natural soils in the eastern region of Morocco

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Abstract
This study aimed to assess the species composition and diversity of arbuscular mycorrhizal fungi (AMF) in two soil samples from two sites in the eastern region of Morocco; Guercif and Zaïo. The results showed that the spore densities of these sites extracted by wet sieving method were very high (279 and 386 spores/10 g of soil in Zaïo and Guercif sites, respectively). The provisional identification test of isolated AMF revealed the presence of 57 AMF species, belonging to five families (Glomeraceae, Gigasporaceae, Acaulosporaceae, Entrophosporaceae and Archaesporaceae). Glomus, Rhizophagus, Funneliformis, Endogone and Acaulospora were the dominant genera. In addition, mycorrhizal potential of both soils was assessed using the "Most Probable Number" (MPN) method. The results revealed that the number of mycorrhizal propagules in Guercif soil were higher than that in Zaïo soil. It was also shown that the frequency and the intensity of root mycorrhization of leeks transplanted in Guercif soil were higher (90% and 74%, respectively) compared to those transplanted in Zaïo soil (56% and 31%, respectively). These results showed that both soils are generally rich in mycorrhiza fungal propagules and have great mycorrhizogenic power, so it would be interesting to isolate and purify fungal strains and to select those that perform well for a given parameter.

Keywords: Arbuscular mycorrhizal fungi, Diversity, Most probable number, Frequency and intensity of mycorrhization

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not possible without better management of interactions between microorganisms and the plants within the agro-
systems (Plenchette et al., 2005; Adesemoye and Kloepper, 2009). One of these interactions is the mycorrhiizae, which are symbiotic associations between fungi and plants that give plants many benefits. AMF, which are considered bio-fertilizers, are a naturel constituent of soil in most ecosystems and can be found in almost all soils in both dry and temperate zones (Pamiske, 2008). The benefits of AMF in forest and agricultural ecosystems are widely recognized. In addition to its contribution to soil aggregation and structural stability (Rillig et al., 2002), AMF were shown to improve mineral nutrition by enhancing the plants ability to uptake mineral nutrients, (Bencherif et al., 2015), especially phosphate (Sally et al., 2003). Moreover, AMF was shown to confer plants protection against abiotic stresses (such as drought, salinity, heavy metals and changes in temperature) (Begum et al., 2019), as well as against pathogens, pests, and parasitic plants (Jung et al., 2012).

Despite the interest and importance of AMF, the use of these microorganisms in agriculture is still limited, mainly due to the incompatibility between the strains introduced and local edaphic characteristics (Dupponnois et al., 2013), causing disappearance of fungi from the injected inoculum. Thus, it is crucial to select native strains, which can adapt to the constraints of the environment.

The aim of our study is to evaluate the diversity and abundance of AMF species in two soils, from two sites in the east of Morocco (Guercif and Zaïo), which would interesting to exploit them in the development of a composite endomycorrhizal inoculum. The choice of these two sites was based on their potential agricultural production, especially the plain of Guercif which was recently exploited, in contrast to the plain of sabra (Zaïo) which has been cultivated for about 50 years. We also aimed, in the present study, to evaluate the mycorrhizogenic potential in order to enhance their native AMF species. Mycorrhizogenic potential was assessed by the "Most Probable Number" or MPN method (Alexander, 1965; Porter, 1979; Wilson and Trinick, 1982; Gianinazzi-Pearson et al., 1985). The MPN bioassay estimate of the number of infective propagules per weight of the tested soil, which reflect the ability of a soil to initiate the formation of mycorrhizal associations from a quantity of inoculum present in the soil as propagules (Plenchette et al., 1989).

**Material and Methods**

**Soil sample**

In this study, natural soil samples were collected from two sites in eastern Morocco with two different agro-pedological conditions: Guercif and Zaïo whose coordinates are (33°58'45"N, 3°15'41"W, Altitude: 688 m, and 34°54'14"N, 2°48'49"W, Altitude: 138 m, respectively). Four random samples were taken from each site at a depth ranging from 0 to 30 cm; and a composite soil sample was produced for each site. Physicochemical characteristics of soils were determined by two laboratories AGRILABO in Fes and LACQ in Meknes (Morocco).

**Spore isolation**

Isolation of AMF spores was carried out by wet sieving using the technique of Gerdemann and Nicolson (Gerdemann and Nicolson, 1963), followed by centrifugation on a sucrose gradient using the method of Giovannetti et al (Giovannetti et al., 1991). 10 g of each soil sample was mixed with 500 ml of tap water in a beaker and stirred vigorously. The soil suspension was then passed through a sieve column made up of two sieves of 500 µm and 50 µm mesh, under a water jet. The 50 µm sieve was collected in distilled water. The resulting spore suspension was then divided into five tubes and centrifuged for 5 min at 9000 rev/min. after discarding the supernatant, a viscosity gradient was created by adding 15 ml of sucrose solution at 60% to each centrifuge tube. The mixture was then rapidly stirred and centrifuged for 4 min at 3000 rev/min. The supernatant containing the spores was filtered and rinsed through a 50 µm sieve with distilled water to remove sucrose. The spores were then recovered with distilled water in a petri dish. Isolated spores were quantified by direct counting under a binocular magnifying glass to estimate the number of spores in 10 g of soil of each sample (spore density). Five repetitions were performed for each extraction.

Appearance frequency of species (A.F.S %) designates the percentage of a morphotype relative to total number of species. A.F.S % = (ns / nT)*100, where ns is the isolated spores number of the species X and nT is the total spores number.

Appearance frequency of genus (A.F.G): designates the percentage of a total spore species of one genus relative to the total spores .A.F.G % = (nG / nT) *100, where nG is the number of spores of the genus X and nT is the total spores number.
**Spore identification**
The extracted spores of the AMF were observed between a slide and a cover slip under the microscope and then photographed. Taxonomic identification of spores down to the species level was based on the size, color, shape, wall structure and hyphal attachments of the spore. The identification of our spores has been carried out using species descriptions provided by the International Collection of Cultures of Arbuscular Vesicular Mycorrhizal Mushrooms (INVAM, 2017) and other appropriate references (Walker and Mize, 1982; Schenk and Perez, 1987; Schenk and Perez, 1990; Morton and Benny, 1990; Morton and Bentivenga, 1994).

**Estimation of the mycorrhizogenic potential of the studied soils**
Mycorrhizal potential of soils was determined by the "Most Probable Number" (MPN) bioassay, which quantify all infective propagules of AMF in a specific soil (Plenchette et al., 1989). The soil samples were dried at room temperature, then sieved through a 2 mm mesh sieve. The resulting soils were diluted with sterilized sand (autoclaved at 120 °C for 30 min). Five dilutions (1/1, 1/4, 1/16, 1/64, 1/256) were made with five replicates. Two weeks old leek (Allium porrum) seedlings were transplanted into 7/7/8 cm plastic pots, previously filled with the substrates from the dilutions (100 g/pot). All the pots were placed in a greenhouse afterward. After 10 weeks of cultivation, the plants were dug up and the root system of each plant was recovered, washed and stained to reveal the presence of mycorrhizal structures, according to the technique described by Phillips and Hayman (Phillips and Hayman, 1970).

Mycorrhizal structures were observed under the microscope. Each root system showing at least one point of infection (penetration of a hypha in the root) was considered to be mycorrhizal. The MPN of propagules was calculated by the following formula:

\[
\log \text{MPN} = (X \log a) - K_{(S,S)},
\]

where "X" is the average number of positives per repetition, "a" is the dilution factor and "K" was determined in the Fisher and Yates table (Fisher and Yates, 1970) as a function of "Y" (The average number of negatives per repeat) and S.

\[
Y = S - X
\]

S is the dilution number

Cochran defines a formula approximating (SE), the standard deviation of log MPN whose distribution is close to the normal distribution (Cochran, 1950):

\[
SE = 0.55 \sqrt{\frac{\log a}{n}}
\]

Where "a" is the dilution factor and "n" is the number of repetitions.

The 95% confidence interval is obtained as follows:

\[
\text{CI (95%)} = \log (\text{MPN}) \pm 2\text{SE}
\]

To find the lower and upper limits, simply divide and multiply the estimated MPN value by Antilog (2SE), respectively.

**Frequency of mycorrhization and intensity of root colonization**
Mycorrhization was estimated according to the method described by Trouvelot et al. (Trouvelot et al., 1986), allowing the calculation of the two parameters below:

Mycorrhization frequency (F): 
\[
F \% = \left(\frac{\text{N} - \text{No}}{\text{N}}\right) \times 100
\]

where N is the number of fragments observed and No is the number of fragments with no evidence of mycorrhization.

Colonization intensity (M%), which expresses the portion of the colonized cortex in relation to the entire root system:

\[
M \% = \left[\left(0.95 \times n5\right) + \left(0.70 \times n4\right) + \left(0.30 \times n3\right) + \left(0.05 \times n2+n1\right)\right]/N,
\]

where "N" : number of fragments observed and n5, n4, n3, n2 and n1 are the numbers of fragments noted 5, 4, 3, 2 and 1 respectively; Class 5 : over 91%, class 4 : from 51% to 91%, class 3 : from 11 to 50 %, class 2 : less than 10%, class 1 : 1% and class 0 : no mycorrhization.

**Statistical analysis**
All data were analysed using the analysis of variance to a single criterion of classification (ANOVA) or Student t-test when appropriate. Data analysis was performed using SPSS version 20 software for Windows. Values of \(p<0.05\) were considered statistically significant.

**Results**
The physicochemical analyses of the two soils, showed that Zaïo soil is a limestone soil, and the Guercif soil have a heavy clay-silt texture. Both
Guercif soil and Zaïo soil were alkaline (pH; 8.3 and 8.1 respectively) and contained very little organic matter (1.04% and 2.34%, respectively), but were rich in potassium (618 ppm and 910 ppm, respectively) (Table 1).

Guercif soil was low in phosphorus (7.27 ppm) and nitrogen (1.33 mg/100 g soil), however, Zaïo soil was high in phosphorus (74.9 ppm) and medium in nitrogen (170 mg/100 g soil).

Table 1: Physicochemical characteristics of Guercif soil and Zaïo soil

| Site       | Guercif | Zaïo |
|------------|---------|------|
| Clay %     | 36.25   | 56   |
| Limes%     | 34.69   | 30   |
| Sands %    | 29.07   | 14   |
| pH         | 8.30    | 8.1  |
| E.C à 25 C° (mmhos/cm) (extract 1/5) | 0.189 | 0.546 |
| Sodium (mg/kg) | 64.00  | 412  |
| Nitrogen mg/100 g of soil | 1.33   | 170  |
| Organic matter (%) | 1.04   | 2.34 |
| Total limestone (%) | traces | 12.2 |
| Phosphorus(ppm: part-per-million) | 7.27   | 74.9 |
| Potassium (ppm) | 618.80 | 910  |
| Calcium (ppm) | 2658.0 | 13700 |
| Magnesium (ppm) | 185.00 | 1289 |

Figure 1: Spore abundance in Guercif and Zaïo soil. Bars with the different letter were significantly different (P < 0.05)

Spore density and richness at the two study sites

The abundance of AMF spores found in the two study sites was 279 per 10 g of Zaïo soil and 386 per 10 g of Guercif soil; they show a significant difference between soils (Figure 1).

Preliminary identifications (Table 2 and Figure 2) showed that the spores isolated from the two sites belonged to 57 species: *Glomus macrocarpum*, *Glomus microcarpum*, *Glomus glomeratulum*, *Rhizophagus intraradices*, *Claroideoglomus lamellosum*, *Rhizophagus fasciculatum*, *Glomus rubiforme*, *Pacispora chimonobambusae*, *Rhizophagus clarus*, *Glomus clavisporum*, *Claroideoglomus claroideum*, *C. etunicatum*, *Glomus albidum*, *Funneliformis constrictum*, *Glomus deserticola*, *Glomus aggregatum*, *Glomus viscosum*, *Funneliformis geosporum*, *Glomus spinuliferum*, *G. boreale*, *G. minutum*, *Glomus sp 1*, *Glomus sp 2*, *Glomus sp 3*, *Glomus sp 4*, *Glomus sp 5*, *Glomus sp 6*, *Glomus sp 7*, *Glomus sp 8*, *Glomus sp 9*, *Acaulospora leavis*, *Acaulospora colombiana*, *Acaulospora capsicula*, *Acaulospora herreriae*, *A. scrobiculata*, *A. mellea*, *A. gedanensis*, *A. foveata*, *A. morrowiae*, *Acaulospora brasiliensis*, *Acaulospora spinosa*, *Acaulospora sp 1*, *Acaulospora sp 2*, *Acaulospora sp 3*, *Acaulospora sp 4*, *Acaulospora sp 5*, *Scutellospora coralloidea*, *Denticulata nigra*, *Scutellospora calospora*, *Claroideoglomus drummondii*, *Pacispora chimonobambusae*, *Gigaspora margarita*, *Glomus coremioiides*, *Entrophospora infrequens* and *Archaeospora trapezi*.

All these species found in the two sites belong to eleven genera (*Glomus*, *Funneliformis*, *Rhizophagus*, *Acaulospora*, *Entrophospora*, *Denticulata*, *Scutellospora*, *Claroideoglomus*, *Archaeospora*, *Gigaspora*, *Pacispora*, and *Endogone*), five families (Glomeraceae, Gigasporaceae, Acaulosporaceae, Entrophosporaceae and Archaeosporaceae) and three Orders (Diversisporales, Glomerales and Archaeasporales).
Table-2: Characteristics of all AMFs isolated from the soils of the two sites (Guercif and Zaïo)

| No. | Species                  | form     | color          | spore size (μm) | Wall size (μm) | Length of the hypha (μm) | Surface of the spore |
|-----|--------------------------|----------|----------------|-----------------|-----------------|--------------------------|----------------------|
| 1   | Glomus macrocarpum       | Globular | Orange brown   | 145             | 1.3             | -                        | Smooth               |
| 2   | Glomus microcarpum       | Globular | Brown          | 84              | 1               | -                        | Grainy               |
| 3   | Glomus glomeratum        | Globular | Brown          | 120             | 2.5             | -                        | Smooth               |
| 4   | *Rhizophagus intraradices* | Globular | Hyaline        | 157             | 6.7             | -                        | Smooth               |
| 5   | *Claroideoglomus lamellosum* | Globular | Orange        | 229             | 4.5             | 81                       | Smooth               |
| 6   | Glomus rubiforme         | Globular | Yellow         | 174             | 2               | 48                       | Smooth               |
| 7   | *Pacispora chimonobambusae* | Sub-globular | Pale yellow | 84              | 2               | -                        | Grainy               |
| 8   | *Rhizophagus clarus*     | Globular | Brown          | 193             | 3               | 174                      | Smooth               |
| 9   | Glomus clavisporum       | Globular | Brown          | 96              | 1               | -                        | Grainy               |
| 10  | Endogone versiformis     | Globular | Brown          | 133             | 1.5             | -                        | Grainy               |
| 11  | Funneliformis mosseae    | Globular | Yellow         | 84              | 1.3             | -                        | Smooth               |
| 12  | *Claroideoglomus claroideum* | Globular | Pale yellow | 84              | 1.5             | -                        | Smooth               |
| 13  | *Claroideoglomus etunicatum* | Globular | Orange        | 104             | 2               | -                        | Smooth               |
| 14  | Glomus albidum           | Globular | Brown          | 133             | 3.3             | -                        | Smooth               |
| 15  | Funneliformis constrictum | Globular | Almost black   | 72              | 1               | -                        | Smooth               |
| 16  | Glomus deserticola       | Globular | Reddish brown  | 96              | 1.3             | -                        | Smooth               |
| 17  | Glomus viscosum          | sub-globular | Pale yellow | 101             | 2.7             | -                        | Smooth               |
| 18  | Funneliformis geosporum  | Globular | Orange brown   | 122             | 8               | -                        | Smooth               |
| 19  | Glomus spinaliferum      | Globular | Yellow         | 72              | 2.5             | -                        | Smooth               |
| 20  | Glomus boreale           | Globular | Black          | 108             | 2.3             | 30                       | Smooth               |
| 21  | Glomus minutum           | Globular | Hyaline        | 169             | 4.2             | 37                       | Smooth               |
| 22  | *Pacispora chimonobambusae* | Globular | Pale yellow | 133             | 3               | -                        | Grainy               |
| 23  | Glomus sp 1              | Globular | Orange brown   | 84              | 2.5             | -                        | Smooth               |
| 24  | Glomus sp 3              | Globular | Brown          | 104             | 1.8             | -                        | Smooth               |
| 25  | *Rhizophagus intraradices* | Globular | Hyaline        | 120             | 7               | 71                       | Smooth               |
| 26  | Glomus sp 4              | Globular | Brown to black | 72              | 1.6             | -                        | Smooth               |
| 27  | Acaulospora capsicula    | Globular | Brown          | 181             | 6               | -                        | Smooth               |
| 28  | Glomus sp 7              | Oval     | Brown          | 96              | 1.3             | -                        | Grainy               |
| 29  | Glomus sp 8              | Oval     | Dark brown to black | 96            | 2.2             | -                        | Smooth               |
| 30  | Glomus versiforme        | Globular | Brown          | 157             | 3               | -                        | Grainy               |
| 31  | Acaulospora laevis       | Globular | Pale brown     | 82              | 3.3             | -                        | Smooth               |
| 32  | Glomus sp 5              | Globular | Orange         | 72              | 1.7             | -                        | Smooth               |
| 33  | Acaulospora colombiana   | Globular | Yellow         | 169             | 2.8             | -                        | Smooth               |
| 34  | Glomus sp 9              | Oval     | Brown          | 101             | 1.5             | -                        | Smooth               |
| 35  | *Rhizophagus intraradices* | Globular | Yellow        | 58              | 0.8             | 23                       | Smooth               |
| 36  | Glomus sp 6              | Globular | Yellow         | 140             | 4               | -                        | Smooth               |
| 37  | Acaulospora herraeae     | Globular | Yellow         | 205             | 7               | -                        | Smooth               |
| 38  | Acaulospora scrobiculata | Globular | Sub-hyaline    | 82              | 0.8             | -                        | Grainy               |
| 39  | Acaulospora mellea       | Globular | Brown-orange   | 120             | 1               | 109                      | Smooth               |
Species of endomycorrhizal fungi such as *Glomus* sp9, *Acaulospora scorbiculata*, *Acaulispora mellea*, *A. gedeanensis*, *A. foveata*, *Glomus viscosum*, *Archaeispora trappei*, *Acaulospora sp4*, *Glomus boreale*, *Glomus sp4*, *Glomus sp5*, *Glomus sp6*, *Glomus sp7*, *Glomus sp8*, *Acaulospora sp3*, *Glomus minutum*, *Acaulospora brasiliensis*, *Acaulospora spinosa*, *Acaulospora marrowiae*, *Acaulospora sp5* and *Glomus aggregatum*, were found in Guercif soil, but not in Zaïo soil. On the other hand, *Acaulospora colombiana*, *Claroideoglomus lamellosum*, *Glomus* sp2, *Claroideoglomus drumondii*, *Glomus rubiforme*, *Acaulospora sp1*, *Scutellospora coralloidea*, *Glomus* sp2, *Acaulospora capsicula*, *Acaulospora herrerae*, were present in Zaïo soil, but not in Guercif soil (Table 3 and Figures 3 and 4).

| No. | Species                     | Shape      | Color         | Spores | Gelatinization | Surface  |
|-----|-----------------------------|------------|---------------|--------|----------------|----------|
| 40  | *Acaulospora gedanensis*   | Globular   | Hyaline       | 72     | 3              | 30       | Smooth   |
| 41  | *Acaulospora foveata*      | Sub-globular | Orange    | 96     | 3              | 31       | Smooth   |
| 42  | *Acaulospora morrowiae*    | Globular   | Yellow       | 108    | 6.4            | -        | Grainy   |
| 43  | *Acaulospora brasiliensis* | Oval       | Yellow       | 84     | 1.6            | 52       | Rough    |
| 44  | *Acaulospora spinosa*      | Globular   | Yellow-brown | 133    | 2.7            | 37       | Thomy    |
| 45  | *Acaulospora sp 1*         | Globular   | Grown        | 133    | 2.5            | 75       | Smooth   |
| 46  | *Rhizophagus intraradices* | Globular   | Hyaline      | 96     | 1.5            | 99       | Smooth   |
| 47  | *Rhizophagus fasciculatum* | Globular   | Brown        | 84     | 3.3            | -        | Smooth   |
| 48  | *Acaulospora sp 2*         | Globular   | Hyaline      | 120    | 0.8            | 22       | Smooth   |
| 49  | *Funneliformis mossaeae*   | Globular   | Yellow       | 91     | 2.8            | -        | Smooth   |
| 50  | *Acaulospora sp 5*         | Globular   | Hyaline      | 84     | 1.5            | 18       | Smooth   |
| 51  | *Scutellospora coralloidea* | Globular   | Yellow       | 174    | 3.5            | -        | Smooth   |
| 52  | *Dentiscutata nigra*       | Globular   | Black        | 133    | 2.6            | -        | Smooth   |
| 53  | *Scutellospora calospora*  | Globular   | Pastel yellow| 165    | 1.5            | -        | Smooth   |
| 54  | *Claroideoglomus drumondii* | Globular   | Hyaline      | 96     | 1.7            | -        | Smooth   |
| 55  | *Acaulospora sp 3*         | Globular   | Yellow       | 72     | 4              | -        | Smooth   |
| 56  | *Gigaspora margarita*      | Globular   | Yellow       | 84     | 1.5            | -        | Smooth   |
| 57  | *Glomus spinuliferum*      | Globular   | Orange       | 229    | 12             | -        | Smooth   |
| 58  | *Funneliformis geosporum*  | sub-globular | Brown-orange| 84     | 1.7            | 36       | Smooth   |
| 59  | *Glomus coremioides*       | Globular   | Brown        | 72     | 2.5            | -        | Grainy   |
| 60  | *Entrophosphospora infrequens* | Globular | Brown-orange | 241    | 4              | -        | Smooth   |
| 61  | *Archaeispora trappei*     | Globular   | Hyaline      | 91     | 1.5            | -        | Smooth   |
| 62  | *Acaulospora sp 4*         | Globular   | Yellow       | 70     | 1.2            | -        | Smooth   |
| 63  | *Glomus multicaule*        | Globular   | Yellow       | 120    | 1              | 108      | Smooth   |
| 64  | *Glomus macrocarpum*       | Globular   | Yellow-brown | 84     | 11             | -        | Smooth   |
| 65  | *Glomus microcarpum*       | Globular   | Brown        | 169    | 4.5            | 61       | Smooth   |
| 66  | *Glomus sp 2*              | Globular   | Hyaline      | 193    | 2.2            | 48       | Smooth   |
| 67  | *Acaulospora laevis*       | Globular   | Yellow       | 104    | 2.5            | -        | Smooth   |
| 68  | *Glomus aggregatum*        | Globular   | Brown        | 96     | 1.5            | -        | Smooth   |
| 69  | *Acaulospora colombiana*   | Ellipsoid  | Orange       | 84     | 1.4            | -        | Smooth   |
| 70  | *Acaulospora scorbiculata* | Globular   | Light brown  | 96     | 3              | -        | Grainy   |
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Figure 2: Photos of endomycorrhizal fungi species found in the soil of two sites in eastern Morocco: Guercif and Zaïo.

*Glomus macrocarpum* (1); *Glomus microcarpum* (2); *Glomus glomerulatum* (3); *Rhizophagus intraradices* (4); *Claroideoglomus lamellosum* (5); *Glomus rubiforme* (6); *Pacispora chimonobambusae* (7); *Rhizophagus clarus* (8); *Glomus clavisporum* (9); *Endogone versiforme* (10); *Funneliformis mosseae* (11); *Claroideoglomus claroideum* (12); *Claroideoglomus etunicatum* (13); *Glomus albidum* (14); *Funneliformis constrictum* (15); *Glomus deserticola* (16); *Glomus viscosum* (17); *Funneliformis geosporum* (18); *Glomus spinuliferum* (19); *Glomus boreale* (20); *Glomus minutum* (21); *Pacispora chimonobambusae* (22); *Glomus sp 1* (23); *Glomus sp3* (24); *Rhizophagus intraradices* (25); *Glomus sp4* (26); *Acaulospora capsicula* (27); *Glomus sp7* (28); *Glomus sp8* (29); *Glomus versiforme* (30); *Acaulospora leavis* (31); *Glomus sp5* (32); *Acaulospora colombiana* (33); *Glomus sp9* (34); *Rhizophagus intraradices* (35); *Glomus sp6* (36); *Acaulospora herreræ* (37); *Acaulospora scrobiculata* (38); *Acaulospora mellea* (39); *Acaulospora gedanensis* (40); *Acaulospora foveata* (41); *Acaulospora morrowiae* (42); *Acaulospora brasiliensis* (43); *Acaulospora spinosa* (44); *Acaulospora sp1* (45); *Rhizophagus intraradices* (46); *Rhizophagus fasciculatum* (47); *Acaulospora sp2* (48); *Funneliformis mosseae* (49); *Acaulospora sp5* (50); *Scutellospora coralloidea* (51); *Dentiscutata nigra* (52); *Scutellospora calospora* (53); *Claroideoglomus drummondii* (54); *Acaulospora sp3* (55); *Gigaspora margarita* (56); *Glomus spinuliferum* (57); *Funneliformis geosporum* (58); *Glomus coremioides* (59); *Entrophospora infrequens* (60); *Archaeospora trappei* (61); *Acaulospora sp4* (62); *Glomus multicaule* (63); *Glomus macrocarpum* (64); *Glomus microcarpum* (65); *Glomus sp2* (66); *Acaulospora laevis* (67); *Glomus aggregatum* (68); *Acaulospora colombiana* (69); *Acaulospora scrobiculata* (70)
Table-3: Appearance of AMF species in soils at study sites (+): Present and (-): Absent

| Species                              | Site Guercif | Site Zaïo |
|--------------------------------------|--------------|-----------|
| Glomus deserticola                   | +            | +         |
| Scutellospora coralloidea            | -            | +         |
| Claroideoglomus drummondii           | -            | +         |
| Rhizophagus intraradices             | +            | +         |
| Scutellospora nigra                  | +            | +         |
| Glomus macrocarpum                   | +            | +         |
| Glomus aggregatum                    | +            | -         |
| Acaulospora scorbiculata             | +            | -         |
| Claroideoglomus lamellosum           | -            | +         |
| Glomus glomerulatum                  | +            | +         |
| Glomus rubiforme                     | -            | +         |
| Acaulospora colombiana               | -            | +         |
| Acaulospora melleea                  | +            | -         |
| Acaulospora gedanensis               | +            | -         |
| Acaulospora foveata                  | +            | -         |
| Glomus viscosum                      | +            | -         |
| Glomus multicaule                    | -            | +         |
| Rhizophagus clarus                   | +            | +         |
| Funneliformis mosseae                | +            | +         |
| Archaeospora trappei                 | +            | -         |
| Acaulospora capsicula                | -            | +         |
| Glomus spinelliforme                 | +            | +         |
| Glomus boreale                       | +            | -         |
| Rhizophagus fasciculatum             | +            | +         |
| Funneliformis mosseae                | +            | +         |
| Glomus sp7                           | +            | -         |
| Glomus sp8                           | +            | -         |
| Claroideoglomus claroides            | -            | +         |
| Endogone versiformis                 | +            | +         |
| Glomus sp3                           | +            | +         |
| Glomus sp2                           | -            | +         |
| Acaulospora sp4                      | +            | -         |
| Glomus sp9                           | +            | -         |
| Glomus sp4                           | +            | -         |
| Glomus sp5                           | +            | -         |
| Claroideoglomus etunicatum           | +            | +         |
| Glomus albidum                       | -            | +         |
| Glomus microcarpum                   | +            | +         |
| Acaulospora sp1                      | -            | +         |
| Acaulospora sp3                      | +            | -         |
| Glomus coremioides                   | -            | +         |
| Glomus sp6                           | +            | -         |
| Glomus minutum                       | +            | -         |
| Acaulospora sp5                      | +            | -         |
| Acaulospora laevis                   | +            | +         |
| Acaulospora brasiiliensis            | +            | -         |
| Acaulospora spinosa                  | +            | -         |
| Glomus clavisporum                   | +            | +         |
| Acaulospora herreriae                | -            | +         |
| Funneliformis constructum            | +            | +         |
| Paccinia sp. chimonobanbasae         | +            | +         |
| Glomus sp1                           | -            | +         |
| Acaulospora sp2                      | -            | +         |
| Gigaspora margarita                  | +            | +         |
| Acaulospora marrowiae                | +            | -         |
| Entrophospora infrequens             | +            | +         |
| Scutellospora calospora              | +            | +         |

Figure-3: Frequency of occurrence of mycorrhizal species in Guercif soil

Figure-4: Frequency of occurrence of mycorrhizal species in Zaïo soil

Figure-5: Frequency of occurrence of the genera of endomycorrhizal fungi at both sites: Guercif (A) and Zaïo (B). Data expressed as mean ± SEM. Values followed by the same lower case letters were not significantly different, whereas values followed by different lower case letters were significantly different (p< 0.05)

In Guercif soil, the genus Glomus presented a significantly higher frequency of occurrence (49.78%) of genera compared to other genus (p<
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0.05), followed by Funneliformis (17.40%), Rhizophagus (11.10%), Acaulospora (10.04%), Entrophospora (3.52%), Dentiscutata (3%), Scutellospora (1.31%), Glomus (1.29%), Acaulospora (1.04%), Endogone (3.52%), Claroideoglomus (0.77%) and Pasispora (0.51%). In Zaïo soil, the genus Glomus presented a significantly higher frequency of occurrence (37.01%) compared to other genus (p < 0.05), followed by Rhizophagus (16.47%), Funneliformis (11.65%), Endogone (8.95%), Claroideoglomus (7.90%), Acaulospora (6.97%), Dentiscutata (4.01%), Pasispora (2.26%), Entrophospora (1.76%), Scutellospora (1.60%) and Gigaspora (1.42%) (Figure 5).

Mycorrhizogenic potential of the studied soils
All leek plants transplanted into Guercif soil, which was raw, diluted 1/4 or 1/16 were mycorrhizal (100% infection). Those transplanted on the highly diluted Guercif soil (1/64 or 1/256) showed an 80% of mycorrhization (Table 4). On the other hand, the percentage of inoculation of leek plants transplanted into Zaïo soil was decreased with increasing soil dilutions. In fact, at the highest dilution of Zaïo soil (1/256), we did not observe any inoculation of leek plants (Table 4).

This result was corroborated by the number of propagules per 100g of soil; where Guercif soil presented a high number of mycorrhizal propagules (99 propagules/100 g of soil), whereas Zaïo soil showed a lower number of propagules (34 propagules/100 g of soil) (Table 5).

Table 4: Number of mycorrhizal plants cultivated on the two soils studied (Guercif and Zaïo)

| Dilutions | site       | Rehearsals | Number of mycorrhizal plants |
|-----------|------------|------------|-----------------------------|
| 1/1       | Guercif    | +          | +                            | +                            | +                            | +                            | +                            | 5                            |
|           | Zaïo       | -          | -                            | -                            | -                            | -                            | -                            | 5                            |
| 1/4       | Guercif    | +          | +                            | +                            | +                            | +                            | +                            | 5                            |
|           | Zaïo       | -          | -                            | -                            | -                            | -                            | -                            | 5                            |
| 1/16      | Guercif    | +          | +                            | +                            | +                            | +                            | +                            | 5                            |
|           | Zaïo       | -          | -                            | -                            | -                            | -                            | -                            | 4                            |
| 1/64      | Guercif    | +          | +                            | +                            | +                            | +                            | -                            | 4                            |
|           | Zaïo       | -          | -                            | -                            | -                            | -                            | -                            | 2                            |
| 1/256     | Guercif    | +          | +                            | +                            | +                            | +                            | -                            | 4                            |
|           | Zaïo       | -          | -                            | -                            | -                            | -                            | -                            | 0                            |

The frequency of mycorrhizal leek roots in Guercif soil was significantly higher (90%) than that in Zaïo soil (56%) (Figure 6). Similarly, the intensity of plant root colonization in Guercif soil reached 74%, and was significantly higher than that in Zaïo soil. The latter did not exceed 31% after 2 months of planting (Figure 6).

Discussion
Our results from this study revealed the richness and the diversity of mycorrhizal fungi in two sites in eastern Morocco: Guercif and Zaïo. While the spore density was high at both sites, it was significantly higher in Guercif soil compared to Zaïo soil (386 and 279 spores per 10 g of soil, respectively). The densities in our study were higher than those reported by different other studies in different areas of Morocco. In fact, previous studies reported spore intensity of 7.8 spores per 10 g of soil in the rhizosphere of *Populus alba* in the northwest of Morocco (Talbi et al., 2014) and 6.3 to 9.8 spores per 10 g of soil in the coastal dunes of Souss-Massa’ region of Morocco (Hatimi and Tahrouch, 2007). On the other hand, spore densities from our study are comparable to those obtained in the maize rhizosphere in the agroecological zone of fisheries in Benin (325.9 per 10 g of soil) (Leslie-Dolorès et al., 2019). However, the spore densities in eastern Morocco in the present study, are noticeably lower than those found in the maize rhizosphere in the food crop agroecological zone south-Borgou in Benin, reaching 1250.15 per 10 g of soil (Leslie-Dolorès et al., 2019).

The variation in spore densities between different sites may result from micro-climatic variations (Koske, 1987), the physico-chemical and/or microbiological properties of soils (Houngnandan et al., 2009). In fact, Panwar and Tarafdar reported that abiotic factors play an important role in the distribution of mycorrhizal fungi (Panwar and Tarafdar, 2006). In our study, the physico-chemical analyses of the samples of the two soils, indicated that both soils were basic (with a pH around 8) with low in organic matter content. In addition, we showed that Zaïo soil contains higher levels of phosphorus and nitrogen compared to Guercif soil. This increase in phosphorus and nitrogen in Zaïo soil is probably the consequence of previous intensive use of mineral fertilizers in this site, which contributes to the degradation of the soil microflora and decreases the number of AMF. On the other hand, the higher spore density in Guercif soil could have been the result of the relatively shorter time of exploitation of Guercif’s plains, thus the lack of intensive fertilizers uses.

Examination of these two soils showed natural existence of a highly diverse community of fungal isolates of AMF. A total of 57 species of arbuscular mycorrhizal fungi belonging to 11 genera and 5 families were isolated and identified. Guercif soil was found to be richer than Zaïo soil in AMF species (42 species vs 36 species, respectively). Variations in the species composition of arbuscular mycorrhizal fungi were observed between the two sites. These differences could be due to the differences in soil composition and the climate between the two sites, given their different geographical locations. Since different fungal species have different functional roles, these results are satisfactory, and this increase in species diversity could translate into an increase in functional diversity.

The count of mycorrhizal fungi spores showed a predominance of the genus *Glomus*, with a frequency of occurrence of 49.78%, represented by 17 species in our samples from Guercif soil. In Zaïo soil, the *Glomus* frequency, represented by 14 species, was 37.01%. It has been reported that the genus *Glomus* is the dominant genus in various natural ecosystems (Hijri et al., 2006). This dominance may be associated with the ability of this genus to produce more spores in a shorter time compared to other genera (Bever et al., 1996), as well as its adaptability to drought and soil salinity (Blaszkowski et al., 2002). For instance, Mosse showed that the genus *Glomus* is often found in neutral or alkaline pH (Mosse, 1973). In Morocco, the dominance of the genus *Glomus* has also been observed in the rhizosphere of olive trees in three regions of Morocco (Tafilalt, Zagora and Taounate) (Kachkouch et al., 2014), Citrus (Artib et al., 2016) and in sites adjacent to phosphate mines (El Gabardi et al., 2019).

The mycorrhizogenic potential, (expressed as MPN) was 99 propagules per 100 g of Guercif soil. This number was lower in Zaïo soil (34 propagules per 100 g of soil). The estimated values for the two soils in our study remain very high compared to those found in other studies. Azcón-Aguilar et al. reported a value of about 24 propagules /100 g of soil in open soil (Azcón-Aguilar et al., 2003). However, Meddich et al. reported higher values which reached 1627 propagules per 100 g in rhizospheric soils of palm groves in tafilalt, Morocco (Meddich et al., 2017).

The mycorrhizal frequency of leek roots transplanted into was significantly higher in Guercif soil (90%) compared to that in Zaïo soil (56%). This variability in the frequency of mycorrhization from one site to another can be explained by differences in the physico-chemical properties of the soils used. For instance, in contrast to Guercif soil which is low in phosphorus (7.27 ppm), Zaïo soil had 10 times more phosphorus content (74.9 ppm). In fact, our results
corroborate several other studies suggesting that mycorrhization frequencies are higher in soils with low total phosphorus levels (Kachkouch et al., 2012). Similar to the mycorrhizal frequency, the colonization intensity of plant roots follows the same trend, with higher colonization intensities in Guercif soil (74%) compared to that in Zaïo soil (31%). The values obtained for the mycorrhizal potential of the soils in our study seem to be correlated with the mycorrhization parameters (frequency and intensity of mycorrhization). It should be noted that the mycorrhizal infectivity potential of a soil depends not only on the number of spores present in the soil, but also on their adaptability and infectivity.

**Conclusion**

The analysis of the richness and diversity of mycorrhizal fungi from two sites in the eastern region of Morocco; Guercif and Zaïo showed that these two prospected soils harbor a variety of AMF communities, represented by 57 species, 42 for the Guercif soil and 36 for the Zaïo soil, dominated by the genus Glomus. The Guercif soil had a spore density of 386 spores per 10 g of soil, a frequency and intensity of root colonization of 90% and 74% respectively, as well as a mycorrhizogenic potential of 99 propagules/100 g of soil which are higher than those found in the Zaïo soil (spore density: 279 spores per 10 g of soil; Frequency of mycorrhization: 56%; Mycorrhization intensity: 31%; Mycorrhizogenic potential: 34 propagules/100 g of soil).

This study characterized, the mycorrhizal fungi naturally present in both soils that seem capable of acting as a source of natural inoculum, and have the potential of being a powerful tool in organic farming practices, which are part of sustainable land management.

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Chafai W: Conceived idea, designed research methodology, conducted experiments, data analysis, literature review and article write up

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Douira A: Literature review, article editing and write up

Khalid A: Conceived idea, final editing and approval of article