Mycorrhizal diversity and root colonization potential of agricultural soils – Doukkala, Morocco

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Abstract— *Underarid and semi-arid ecosystems where drought, soil salinity and low soil fertility considerably limit crop production. Under such stressful growing conditions, an appropriate management of mycorrhizae may have a determinant impact on plant production, on the improvement of soil quality and the diversification of cultivated crops. In this context, the diversity of the arbuscularmycorrhizae flora in semi-arid agricultural soils of OuledGhanem and SidiBennour sites of the Doukkala region, Morocco was evaluated and the impact of soil physico-chemical factors on their root-colonization potential studied. The two selected studied sites are respectively characterised by neutral to alkaline substrates, low level of organic matter but differed in their respective texture, sandy soil and muddy soil and their low to medium available P contents. The comparative analysis of the arbuscular mycorrhizal flora reveals a certain amount of shared species and is characterized in sandy soils by a good proportion of Diversisporales: Scutellosporacalospora, Acaulosporacavernata, Entrophosporainfrequens, Gigaspora margarita when the muddy soils are inhabited with a majority of Glomerales (Glomus.sp). Correlation between physico-chemical and biological soil data enlighted the negative impact of alkalinity and available phosphorus on the soil root colonization potential. The comparative data analyses bring in a critical look at the possible interaction between fungal diversity, mycotrophic plant and root colonization.*

Keywords— Arbuscular, mycorrhiza,biodiversity, Glomeromycetes, mycotrophic.

I. INTRODUCTION

The global phenomenon of soil degradation affects a large percentage of agricultural territories. Following in the impact of natural and human factors, this deterioration is reflected by a Disruptionof the functioning of ecosystems, including a decline in crop yields and a reduction in biodiversity [1]. The agricultural Doukkala region of southern Morocco with semi-arid climate, is affected by this degradation, because of severe climatic conditions aggravated by many human activities which that seriously weaken the soil.

The evaluation of soil quality rests on the choice of appropriate indicators and the methods of analysis which vary according to the sites, the climates and the substrates studied. Most of the methods are based on the physical-chemical analysis, however, during the last decade; the interest at the soil microflora has highlighted the essential role played by these organisms, both at the level of the quality of the soil, the nutrition and growth of cultivated plants. This approach aims to assess the quality of the soil with bio-indicators, such as the microbial biomass, some enzymatic activities and the colonization mycorhizal of roots [2], [3], [4].

The arbuscularmycorrhizal fungi(AM) live in symbiosis with a majority of cultivated plants. Their beneficial effects are reflected by a better growth and increased protection against various biotic and abiotic stresses. In soils, the mycorrhizae will lead to the diversification of the telluric microflora which limited their gradual impoverishment resulting from successive monoculture and intensive fertilizations. In addition, mycorrhizal networks once well established improve the stability of the soil through a better aggregation [5]. The exploitation of this symbiosis is therefore interesting for the soils of arid and semi-arid areas where drought and salinity are limiting factors [6]. Their benefit is also located at the level of the protection of crops against some parasites's roots [7], [8]. These profits on plant production and on the maintenance of the quality of the cultivated soils have triggered many researches on their diversity in the soil, their spread and their preservation for use as inoculums.

The present work aims to explore the diversity ofthe arbuscularmycorrhizal florain the agricultural soils of two areain the region of the Doukkala, and to assess their potential for colonization on root plant trap in laboratory, using a standard bioassay: the biotest[9].

II. MATERIAL AND METHODS

2.1 Soils samples

The Doukkala region is characterized by a semi-arid climate with oceanic influence. The soils sampled from two different areas:

- Coastal area: Region of OuledGhanem, (GH) in the sandy soil cultivated with cereals (GH1) and with potato (GH2)
- Continental area: Region of SidiBennour (SB) in the ground of silty type (Faid) grown in sugar beet at the time of sampling (SB1) and after rotation of one year with cereals.

Soil sampling and samples preparation for the Biotest are carried out according to the methods RM-ERL-2, B-M-PN and B-PAL [9]. The soil samples were sieved to 2 mm, and kept at 4°C. The rates of organic matter and of Phosphorusassimilated have been determined respectively by the methods of Walkey and Black [10] and Olsen [11].

2.2 Biotest

The sub-soil samples sieved have been deposited in plastic pots 400 mL (9 cm of diameter) and each pot planting 15 seeds of leek (*Allium porrum L.*), with 10 repetitions per site sampled. The controls soils have been sterilized by autoclaving (180°C for 2 hours). During the phase of germination, the soil is kept moist and the plants pruned after 2-3 weeks of growth to retain only 4 plants per pot. After two months of culture, the roots collected have been washed, bleached, stained and observed under the microscope, according to the method of Philips and Hayman [12]. The rate of root colonization (hyphae, vesicle and arbuscular) was evaluated by applying the method B-M1P [9].

2.3 Extraction and identification of spores

After wet sieving (sieves of 300, 150 and 38 µm) of 50 g of the soil, followed by a sucrose

Gradient[13], the supernatant was filtered under vacuum on paper Whatman no. 2. The spores were extracted manually under magnifying glass and mounted in Polyvinyl Lactic Acid Glycerol (PVLG)[14] and a 1:1 (PVLG-Melzer's reagent). The identification of species has been carried out with the original descriptions of key

electronic synoptic and comparisons with type specimens: (http://invam.caf.wvu.edu/fungi/taxonomy/speciesid.htm http://www.agro.ar.szczecin.pl/~jblaszkowski/index.html

2.4 Statistical analysis:

An analysis of variance (ANOVA1) by the statistical software "SPSS V. 10" has been performed on the percentages of the different variables transformed according to the formula: 2arcsin √ value in %.

The means comparisons of the variables measured in the different sites has been carried out with the test Duncan.

III. RESULTS AND DISCUSSION

3.1 Physico-chemical analysis

The soils of two sites are characterized by a rate of organic matter negligible with respectively 1.24 g and 1.44 g/ 100g for SidiBennour (SB) and OuledGhanem (GH).

The statistical analysis "Fig 1" shows that there is no significant difference in the composition in organic material between the soils of two sites. By contrast, the data of pH and P assimilated present differences highly significant; the soils of SB are characterized by a pH close to neutrality (7.69) and a rate of P (26.14 µg/g) while those of GH by a pH basic (8.20) and a rate of P (102.1µg/g).

FIGURE 1. COMPARISON OF PHYSICO-CHEMICAL OF THE TWO SITES LEGEND.NS: DIFFERENCE NOT SIGNIFICANT, * DIFFERENCE VERY HIGHLY SIGNIFICANT**

3.2 ArbuscularMycorrhizal Flora

Ten species of AM fungi have been found for each site. Five species are common to the two sites are *Glomus mosseae*, *Glomusmacrocarpum*, *Glomusintraradices*, *Glomusverruculosum* and *Pacisporascintillans*.Overall, the species of the genus *Glomus* dominate with 5 species found for the site GH "Table 1" and 8 species for the site SB "Table 2".

TABLE 1
AM FUNGALSPECIES IDENTIFIED IN SUB-SAMPLES OF OULEDGHANEM (GH)

Fungi species	GH1-1	GH1-2	GH1-3	GH1-4	GH2-1	GH2-2	GH2-3	GH2-4
Glomus mosseae	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
G. macrocarpum	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
G. intraradices	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
G. albidum		**X**						
G. verruculosum					**X**		**X**	
Gigasporamargarita		**X**						
Scutellosporacalospora	**X**			**X**				
Acaulosporacavernata							**X**	
Entrophosporainfrequens		**X**						
Pacisporascintillans		**X**						
Total species	4	7	3	4	4	3	5	3

The distribution profile of species varies considerably from one site to another. In the site GH, we found several species of the Diversisporalesorder:*Scutellosporaacalospora, Acaulosporacavernata*, *Entrophosporainfrequens*, *Gigaspora margarita* as well as the species *Glomusalbidum*whereas the cohort of site SB is composed only of representatives of the Glomerales: *Glomusrubiforme, Glomusconstrictum*, *Glomuslamellosum*, *GlomuscoronatumandGlomus sp.* with white spores which the species is not identified.

Similarly, the species composition varies considerably in the sub-samples of a same site. The sub-samples of the site GH contain between 3 to 7 species, *Glomusmosseae*, *Glomusmacrocarpum*,*Glomusintraradices*, being present in all sub-samples while the other only appear occasionally. The sub-samples of site SB, contain between 2 to 9 species, *Glomusmosseae* and *Glomusmacrocarpum* being common to all sub-samples. Although the site SB either cultivated by a non mycotrophic plant ,

sugar beet, in rotation of one year with a mycotrophic plant, the flora remains active and the diversity in AM fungi is important as in the soils of the site GH cultivated by mycotrophic plants. The two sites have a total of 10 species listed, representatives of Glomeraceae (Glomerales) with the exception of *P.scintillans* (Diversisporales),

An abundant spore population of *P. scintillans* species were isolated from soils of both sites and considered a first record for North Africa countries.

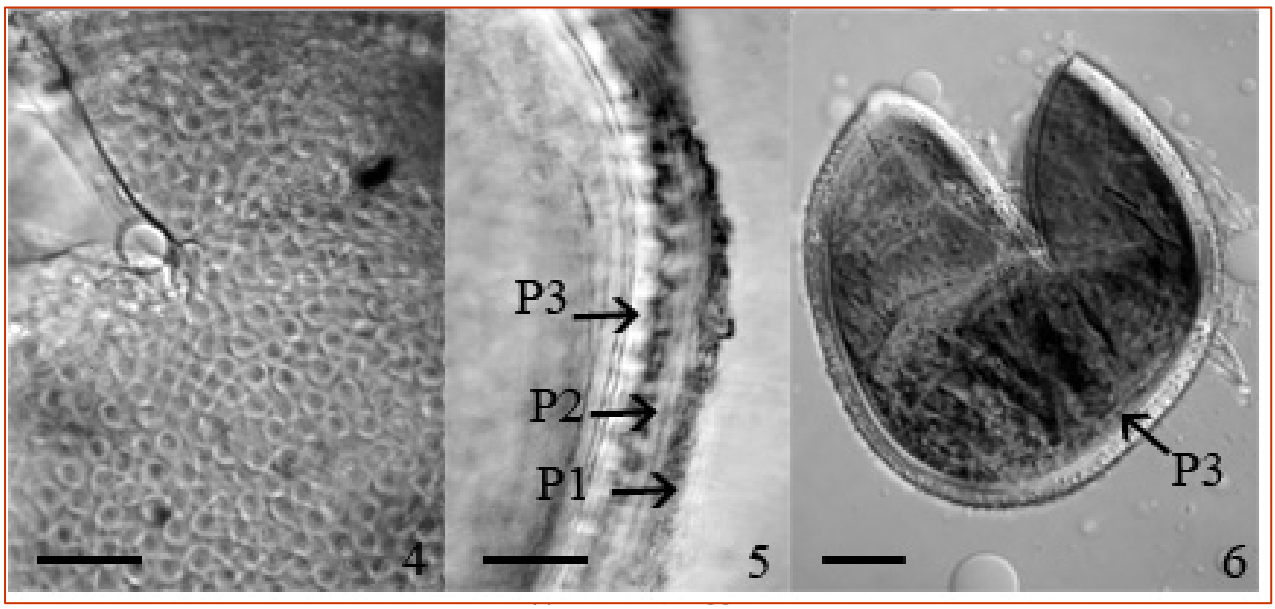
The species *Glomusrubiforme*, *Glomusconstrictum*, *Glomuslamellosum*, *Glomuscoronarum* and *Glomus sp*. constitute the cohort specific of site SB.

TABLE 2

AM FUNGAL SPECIES IDENTIFIED IN SUB-SAMPLES OF SIDIBENNOUR (SB)

Fungi species	SB1-1	SB1-2	SB1-3	SB 1-4	SB 2-1	SB 2-2	SB 2-3	SB 2-4
G. mosseae	**X**	**X**	**X**	**X**		**X**	**X**	**X**
G. macrocarpum	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
G. intraradices	**X**	**X**	**X**		**X**		**X**	**X**
G. rubiforme						**X**	**X**	**X**
G. constrictum			**X**		**X**		**X**	**X**
G. verruculosum		**X**						
G. lamellosum		**X**					**X**	
G. coronatum		**X**					**X**	
Pacisporascintillans					**X**		**X**	**X**
*Glomus*sp							**X**	
Total species	3	6	4	2	4	3	9	6

The specie not identified *Glomus sp.* is not apparent in any of the species currently described “Fig. 2-6”. It is distinguished by ellipsoid toamygdaloïdspores, 110-115 X 80-90 µm diam. white to yellow color “Fig. 2”, with a thickness wall 8.5 -10 µm, composed of three layers “Fig. 5”: P1, vitreous, < 1µm, P2, vitreous to white, 8- 9.5 µm, unitary, adorned with the internal surface of invaginations, regular appearing in surface of spore as adjacent craters of 2.4 - 3.1 µm in diameter, P 3, pale yellow, 2.3-3.7 µm, reactivated in russet in Melzer medium “Fig. 6”.Hyphae has a cylindrical suspensor, 13-16µm in diameter, wall 6-8µm thick “Fig.3”. The attempts to put in culture of the strain *Glomussp.* have not yet allowed generating its culture.



FIGURES 2-6: MORPHOLOGICAL CHARACTERISTICS OF SPORES OF GLOMUS SP.FIGURE 2: SPORE FULL. FIGURE 3: SUSPENSOR HYPHA. FIGURE 4: DETAILS OF ORNAMENTATIONS. FIGURE 5: WALL OF THE SPORE: EXTERNAL WALL (P1), MID-WALL UNITARY ORNAMENTED (P2), INTERNAL LAMINATE WALL (P3). FIGURE 6: SPORE OVERWRITTEN WITH INTERNAL WALL REACTIVATED AT MELZER.

(Ladders: Figures. 2 and 6 ▬▬ =20µm. Figures.3-5 ▬=10µm.)

3.3 Biotest

The roots of the leek cultivated in soils of SB have a significantly higher percentage of intraradical arbuscules and vesicles than those grown in the soils of GH "Fig.7". However, the differences are not significant when the comparison is performed by measuring the percentage of hyphae colonizing these roots. Generally, the rate of colonization of roots from soils of SB that are poor in phosphorus is significantly higher than that obtained with the soils of GH more rich in phosphorus.

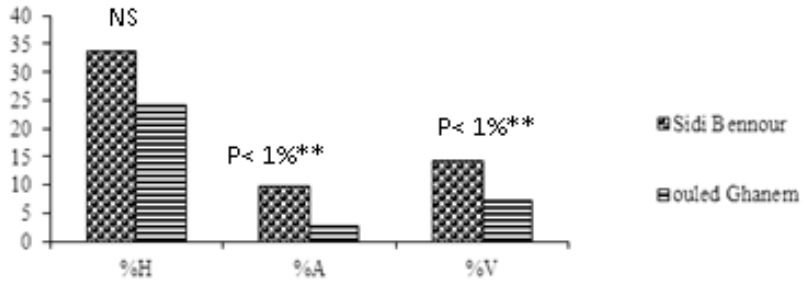


FIGURE 7: COMPARISON OF RATES OF ROOT COLONIZATION OBTAINED WITH THE SOILS OF THE TWO SITES STUDIED.

*Legend: NS: Difference not significant, ** Differences highly significant*

%H: Percentage of hyphae, %A: Percentage of arbuscular, %V: Percentage of vesicles

The physico-chemical analyzes show a alkalinity of sandy soil (GH) significantly more basic than the silty soils (SB) [15]. We can link this alkalinity either to the nature of the parent rock, rich in limestone, or to enrichment in cations (Ca^{2+}, Mg^{2+} and Na^{+}) of marine origin due to the proximity of the sea. The low rate of organic matter, usually less than 1.5 % [16] is due to a poor management of crop residues and of a crop intensification [17], [16].The rate of P equated higher at site GH is possibly related to a higher contribution of fertilizer, to the low adherence of this element to the colloids of soils related to its sand texture and to its low organic matter content that makes it more available to the roots.

In terms of AMspecies diversity, the soils of the two sites have a diverse microflora represented by ten species including a majority of spores belonging to the genera *Glomus* and *Pacispora*. The most frequent species, *Glomusmosseae*,

*Glomusmacrocarpum and Glomusintraradices*found in almost all the sub-samples of the two sites studied, without regard to the mycorrhizal status of plants cultivated. Whether it be soil habitats of arid, semi-arid, temperate or arctic, these three species have been regularly inventoried in soils native or cultivated from many ecosystems and climates, [18], [19], [20], [21], [22], [23].As to the companionspecies, their taxonomic diversity, significantly higher than in the soils of the site GH, is reflected by the presence of representatives of the genera *Glomus*, *Gigaspora*, *Acaulospora*,*Entrophospora*and *Pacispora*, whereas this diversity is confined to the genera *Glomus* and *Pacispora*in the soils of the site SB. A few previous inventories in arid and semi-arid areas however indicate a predominance of species of the genus *Glomus* which seem well adapted to the water stress and saline [19],[21], [24], [25], [26].

The correlation between the physico-chemical analyzes and biological diversity indicates the negative effect of the alkalinity and high rate in phosphorous on root colonization "Fig. 7". Under the same physico-chemical conditions, the difference not significant of the percentage of hyphae colonizing the roots indicates that neither the pH, neither the rate of P do seem to affect the growth hyphae in these soils. The majority of the exchanges nutrients between partners are carried out at the level of arbuscularramifications and the functionality of the mycorrhizae therefore depend mainly of the good the settlement of arbuscular intracellular [27]. These results agree with those of numerous experiments demonstrating a net reduction of the rate of mycorrhizae in presence of richer soils in phosphorus and this in relation with various stages of root colonization: growth of external hyphae [22] [28]. Penetration of hyphae in the root [29] and arbuscular differentiation [30].However, the extremely low levels of phosphorus inhibit the root colonization as well as any mycorrhizal activity [31].The agricultural practices of tillage and fertilization will cause over the years a transformation if not a significant reduction in the diversity of AM fungi [32], [33], [34] with the gradual installation of species to mutualistic properties impaired and even parasites [35].The soils of the site GH, grown in rotation of mycotrophic plants (cereals and potatoes), contain a mycorhizal flora diversified with representatives of several genus of Glomeromycetes (*Acaulospora* and *Entrophospora*, *Gigaspora*, *Glomus*, *Pacispora*) whereas the flora observed at site SB, grown in rotation of mycotrophic plants and non-mycotrophic,contains mainly spores of the genus *Glomus*.Although there is no specificity host-fungus in the arbuscularmycorrhizal symbiosis, the existence of an ecological specificity as highlighted by Gollotte et al. [36] in grassland soils that is expressed through the variations of the biodiversity of the mycorrhizal profile. In natural environments, the plants should be developed in harmony with the microorganisms naturally present; the situation is different in the cultivated soils.The physico-chemical characteristics of the soil and the cultural practices such as fertilization and tillage directly affect the microbial and the mycorrhizal activity in cultivated soil as well as its biological diversity [18], [37], [21].The comparative analysis of the mycorrhizas populations of cultivated soil has allows highlighting the presence, the diversity and the interaction of mycorrhizae with other plants and the components physico-chemical soil, notably the Phosphorus and the PH. In addition, the presence of original species such as that of the *Glomus* sp in site SB implies that elements of the mycorrhizal flora might be endemic to the habitats semi-arid. As highlighted in this work, the marked differences in the generic profile in fungi M.A between these soils (*Glomeraceae*, *Gigasporaceae* and *Acaulosporaceae*) leads to the assumption of a possible relationship more closely than expected between the composition of mycorrhizal soil and its physico-chemical properties.

IV. CONCLUSION

The challenge of achieving sustainable crop management, such as those practiced in the area of Doukkala , involves maintaining and improving the efficiency of existing mycorrhizal flora.

To do this, cultural practices without plowing to maintain soil mycelial functional networks, building to the soil plant residues to gradually increase the rate of organic matter and crop rotation favoring longer cycles of mycotrophic plants are easy and inexpensive procedures to be introduced.

On a more fundamental side, isolation, propagation and evaluation of the mycorrhizal potential of each strain listed at poor environment in phosphorus would determine which species contribute advantageously to plant symbiosis.Once identified performance strains, their wide spread and their subsequent incorporation into agricultural soils with low yields located in similar climates would reestablish an effective flora and thus gradually overcome the nutritional deficiencies and increase the resistance of plants to water stress.

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