Impact of crop rotation on mycorrhizal fungi in irrigated soils of the Doukkala (Morocco)

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Abstract— *This study has been conducted on the soils of irrigated perimeter of Doukkala. It is to assess the impact of the rotation of a species not mycotrophic (Beta vulgaris) with cereals on the natural resources of the mycorrhizal arbuscular fungi whose profits for the growth of plants are known. The results obtained show that this type of culture has a negative effect on the richness of the soil in spores and diversity of mycorrhizal fungi, and on the content of arbuscules which are the places of exchanges between the partners. The authors offer recommendations on cultivation practices which can be modulated in order to preserve this natural resource.*

Keywords— Arbuscular mycorrhizal fungi, cultural history, Doukkala, irrigated areas, semi-arid.

I. INTRODUCTION

In Morocco, the irrationalisation of cropping practices, agricultural intensification, inappropriate exploitation of non-renewable natural resources and irrigation are the origin of many soil degradation processes, which are likely to limit their productivity and hinder a sustainable management of this natural resource. Indeed, the work of (Naman et al., 2001) on soils in irrigated areas semi-arid, with agricultural intensification, has highlighted an important decrease of the organic matter and nitrogen component. In addition, Badraoui and al., (1998) have demonstrated that irrigation in the semi-arid areas caused a significant increase of the electrical conductivity annual rate, an increase in the exchangeable sodium and a salinization of soils. In the irrigated perimeter of the Doukkala, despite the efforts made by the guardianship organisms especially after the introduction of the sugar beet, the intensification of the development of agricultural land has generated a deterioration of the quality of the soil and groundwater (Naman and Soudi, 1999; Rahoui et al., 1999; Naman et al., 2001; Naman, 2003). As well, the major worry concern is no longer only the improvement and stabilization of yields, but also the preservation and improvement of the quality of soils; the only guarantee of a sustainable agriculture.

This problem has had the merit of implementing several methods of analysis mainly physico-chemical to assess the state of health of soils. However, the biological component which plays a vital role in the process of mineralization and transformation of organic matter (Chaussod, 1996) has been poorly studied in the soils degraded of semi-arid Moroccan (Nouaim, 1994; Naman, 2003). Thus, the microbial biomass of the soil, mainly the mycorrhizal fungi that form symbioses with the majority of plant species with a few exceptions such as the beet, it played an important role in the transfer of the mineral elements to the plant (Boudarga, 1989; Chaussod, 1996). In effect, the mycelium of these fungi may exceed tens of kilometers per gram of soil and thus represent an extensive trail allowing the flow of nutrients through the soil to the host plant that benefit as well of mineral elements located beyond the area of exhaustion. The researchs of Brundett (1991), Requena et al., (1996), Graf (2004) and Planchette (2005) have demonstrated that these fungi have a very important role in the aggregation and regeneration of semi-arid soils. In addition, it recognizes their role bio-fertilizer (Gianinazzi-Pearson and Gianinazzi, 1986; Strulu, 1991; Müller A.M.et al., 2013), bio-protector (Sylvia and Williams, 1992; Charest et al., 1993; Paradis et al., 1995) and their participation in the regulation of the pool of carbon in the atmosphere and in the soil (Söderström, 2002). It is therefore biological agents, which represent an important component of fertility (Müller et al., 2013), and allow improving the quality of organic soils while ensuring the sustainability of agrosystem.

Except our preliminary work on the mycorrhizae of agricultural soils in the Doukkala area (Boudarga 2004; 2007; 2009) who study the richness in mycorrhizal fungi, we had no reference concerning the biological quality of these soils and more particularly the mycorrhizae which are today considered as biological indicators of the agricultural soils quality (Chaussod, 1996; Kling and Jakobsen, 1998; Egli, 2001).

The aim of this work is to study the impact of sugar beet culture on the richness and the mycorrhizal potential of these soils. The beet is a not mycotrophic plant cultivated in the irrigated area of the semi-arid zone of the Doukkala,The evaluation will

focus on the quantification of the number of spores as well as their identification and on the mycorrhizal activity in the root system of cultivated plants. In parallel, and with the aim of having a control to observe the effect of the culture of the beet on the biodiversity of mycorrhizal fungi arbuscular, a study on the diversity in these fungi is carried out in a region not irrigated (bour) never cultivated by the beet.

II. MATERIAL AND METHODS

2.1 Presentation of the region

The region of Doukkala-Abda is located in the central party oceanic between two major rivers: Oum Rbia and Tensift. The climate is semi-arid; precipitation may vary according to the regions of 140 mm to 650 mm per year. The present work has focused on irrigated plots of the region of Gharbia (GH) and Zemmamra, (ZE) and plots not irrigated in the region of Ouled Ghanem (OG).

2.2 Sampling

2.2.1 Soils

In order to highlight the impact of the beet crop in irrigated areas on the infectious potential of soils of Gharbia and Zemmamra, we chose two types of plots with two background cropping: cereal-cereal (Agricultural history 1) or cereal-beet (Agricultural history 2). For the area of Ouled Ghanem, we have carried out the sampling in the plots not irrigated never cultivated by the beet.

2.2.2 Root System

In order to highlight the potential of functioning of the mycorrhizal symbiosis, we sampled plants cereals with their root system in the two regions (GH, ZE). These plants are cultivated in the same plots already sampled. The assessment was made on 30 fragments of 1cm long for each plant to either the 90 fragments by agricultural history and by region.

2.3 Isolation, quantification and identification of spores

After wet sieving (sieve of 300, 150 and 38 µm) of 50g of the soil followed by a sucrose gradient, the supernatant was filtered under vacuum through Whatman paper no. 2. The quantification of the number of spores was carried out under the Magnifying glass using a petri dish grid.

After wet sieving (sieves of 300, 150 and 38 µm) of 50 g of the soil, followed by a sucroseGradient(Dalpé& Hamel 2007), the supernatant was filtered under vacuum on paper Whatman no. 2. The spores were extracted manually under magnifying glass and mounted in the Polylvinyl alcohol, lactic acid, glycerin (PVLG) (Omar et al. 1979).

The identification of species has been carried out with the original descriptions of key electronic synoptic

http://invam.caf.wvu.edu/fungi/taxonomy/speciesID.htm

http://www.agro.ar.szczecin.pl/~jblaszkowski/index.html

2.4 Evaluation of the functioning of the symbiosis

Evaluation of the functioning of the mycorrhizal symbiosis has been carried out according to the method of Trouvelot and al. (1986) after staining of roots according to the technique of Phillips and Hayman (1970). This method is based on the measurement of the following parameters: Frequency of colonization (F), intensity of colonization (M), content of arbuscules in the area of root with mycorrhizae (a) and content of arbuscules of the global root system (A). This last parameter is a component of the 3 previous and in principle reflect the better the potential for exchanges symbiotic.

2.5 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

The results are shown by region based on the cultural history.

3.1 Region Gharbia "Table 1".

Number of spores:

In the region of Gharbia, we find that the most spores dominant for both types of Agricultural history are those whose size between 50-100µm. The number of spores varies according to cultural history; it falls 1236.67 spores / 100 g soil for the history 2 and 2032.89 spores / 100g of soil in the case of the history1. The comparisons of means by the test t show that soils from plots cultural history cereal-cereal are richer in spores of mycorrhizal fungi and differences are highly significant for the total number of spores ($t = 12.27$, $P < 0.001$).

TABLE 1

COMPARISON BETWEEN THE TWO AGRICULTURAL HISTORIES IN GHARBIA REGION.

	Number of spores / 100g of soil. n ± sd			Potentiality of operation of the symbiosis			
	Ø>100 µm	50< Ø<100 µm	Total	F %	M%	a%	A%
agriculturalHistory 1	199.22±26.30	2032.89±42.84	2232.11±68.96	82.22±0.11	48.53±0.069	72.91±0.047	35.00±0.044
agriculturalHistory 2	94.4±12.81	1236.67±30.55	1331.11±25.11	85.55±0.048	44.36±0.076	34.54±0.012	17.14±0.009
Test t	3.58*	15.13***	12.27***	0.14 NS	0.40 NS	7.30**	4.35*

Analysis of Roots:

The analysis show that for the mycorrhizal frequency (F) there is no significant difference between agricultural histories 1 and 2, the percentages are 82.22% and 85.55% respectively. Similarly, we obtained the same result for the intensity of mycorrhizal (M), 48.53% is noted for the agricultural history 1 and 44.36% for the second.

By against, the percentage of the content of the arbuscular mycorrhizal part (a) for the agricultural history 1 (72.91%) is significantly higher than that of the agricultural history 2 which is 34.54% ($t = 7.30$, $P < 0.01$). In terms of the root system arbuscular content (A), the percentage is 35.00% in the case of agricultural1. There is significantly higher than that of the agricultural history 2 (17.14%). The comparisons of means showed that the roots of plants from agricultural history 1 plots are richer in arbuscular ($t = 4.35$, $P < 0.05$).

We also highlighted the existence of a significant correlation between the number of spores and the content arbuscular roots.

3.2 Zemmamra region "Table 2".

Number of spores:

The number of spores whose size is greater than 100 µm shows no significant difference between the two agricultural histories (270 for history 1 and 247.77 for the history 2). But, spores whose size is between 50-100µm are more abundant in the case of the agricultural history 1 (1661.11 / 100 g of soil) than for the history 2 (863.33 / 100 g of soil). For the total number of spores of all sizes, found that soils from plots history cereal-cereal are richer and comparisons of means shows that the differences are highly significant ($t = 12.332$, $P < 0.001$).

Analysis of Roots:

Regarding the factor (F), the results are comparable for the two agricultural histories. But the factor (M) we found that it is 33.05% for the agricultural history 1 and 23.55% for the agricultural history 2 two, with a highly significant difference ($t = 4.832$, $0.01 < P < 0.05$) "Table 2".

TABLE 2

COMPARISON BETWEEN THE TWO AGRICULTURAL HISTORIES IN ZEMMAMRA REGION

	Number of spores / 100g of soil. n ± sd			Potentiality of operation of the symbiosis			
	Ø>100 µm	50< Ø<100 µm	Total	F %	M%	a%	A%
agriculturalHistory 1	270.00±28.35	1661.11±29.20	1931.11±54.61	78.88±0.040	33.05±0.010	61.40±0.017	20.30±0.010
agriculturalHistory 2	247.77±19.27	863.33±24.03	1111.11±38.02	59.99±0.077	23.55±0.016	39.82±0.067	09.58±0.021
Test t	0.648 NS	21.090 ***	12.332 ***	2.202 NS	4.832 **	3.062 *	3.880 *

Legend: *: Significant difference, **: highly significant difference, *** very highly significant difference, NS: no significant difference, F: mycorrhizal frequency, M: mycorrhizal intensity, a: arbuscules content of mycorrhizal part root, A: arbuscular content of the root system.

Percentages of arbuscules (a) and (A) in plots with agricultural history 1 are almost double those found for the second history. Indeed, the factor (a) is 61.40% for of the agricultural history 1 and 39.82% for the second with significant difference ($t = 3.062$, $P < 0.05$). Similarly, for the parameter (A) plots of the antecedent 1 have a percentage of 20.30% and those of agricultural history 2 with a low percentage 9.58 %. Comparing of the means issignificant ($t = 3.880$, $0.01 < P < 0.05$).We also highlighted the existence of a significant correlation between the number of spores and the content arbuscular roots.

Comparison between the two regions

For spores whose size is greater than 100 µm, the differences between the two regions are not significant in the case of agricultural history 1, but they are in the case of agricultural history 2, with greater richness in the zemmamra region in which it was discovered a value of 247 spores/100 g against 94 spores / 100g in Gharbia

For spores whose size is between 50-100µm, as well as the total number of spores, the differences between the two regions are significant for both agricultural histories, with the highest values in the Gharbia region “Table 3”.

TABLE 3

COMPARISON BETWEEN THE TWO REGIONS BY TYPE OF AGRICULTURAL HISTORY

	Number of spores / 100g of soil			**Potentiality of operation of the symbiosis**			
	Ø>100 µm	50< Ø<100 µm	Total	F %	M%	a%	A%
agricultural History 1	t = 1.830 NS	t = 7.169 **	t = 3.422 *	t = 0.474 NS	t = 2.230 NS	t = 2.235 NS	t = 3.486 *
agricultural History 2	t = 6.625 **	t = 9.604 **	t = 4.828 **	t = 2.846 NS	t = 2.769 NS	t = 1.193 NS	t = 0.174NS

Legend: NS: nonsignificant difference, *: significant difference. **: Highly significant difference, F: mycorrhizal frequency, M: mycorrhizal intensity,a: content of arbuscular mycorrhizal part of the root, A: arbuscular content of the root system

Finally, the parameters expressing the activity of the symbiosis (F, M, a and A), we have found no significant difference between the two regions, except for the factor A in the case of the agriculturalhistory1.

Mycorrhizal arbuscular flora

After identification of species in the three regions (GH, ZE, OG), two species are common to all sites are *G. mosseae*, *G. macrocarpum* irrespective of the region or of the previous harvest "Table 4".

TABLE 4: AM FUNGAL SPECIES IDENTIFIED IN OG, ZE1, ZE2, GH1 ET GH2

Fungi species	OG Cereal	ZE1 Cereal	GH1 Cereal	ZE2 Beet	GH2 Beet
Glomus intraradices	X	X		X	
G. macrocarpum	X	X	X	X	X
G. mosseae	X	X	X	X	X
G. verruculosum	X				
G.rubiforme		X	X	X	
G.albidum	X	X			
G.constrictum			X		
G.coronatum		X	X		
Pacispora scintillans	X	X	X	X	
Acaulospora cavernata	X				
Entrophospora infrequens	X				
Gigaspora margarita	X				
Scutellosporacalospora	X				
Total species	10	7	6	5	2

FIGURE 1: SPORE POPULATION OF P. SCINTILLANS SPECIES. A. SPORE FORMED SINGLY IN SOIL, B AND C. PLAN VIEW OF KNOBBY ORNAMENTATION ON THE SPORE SURFACE

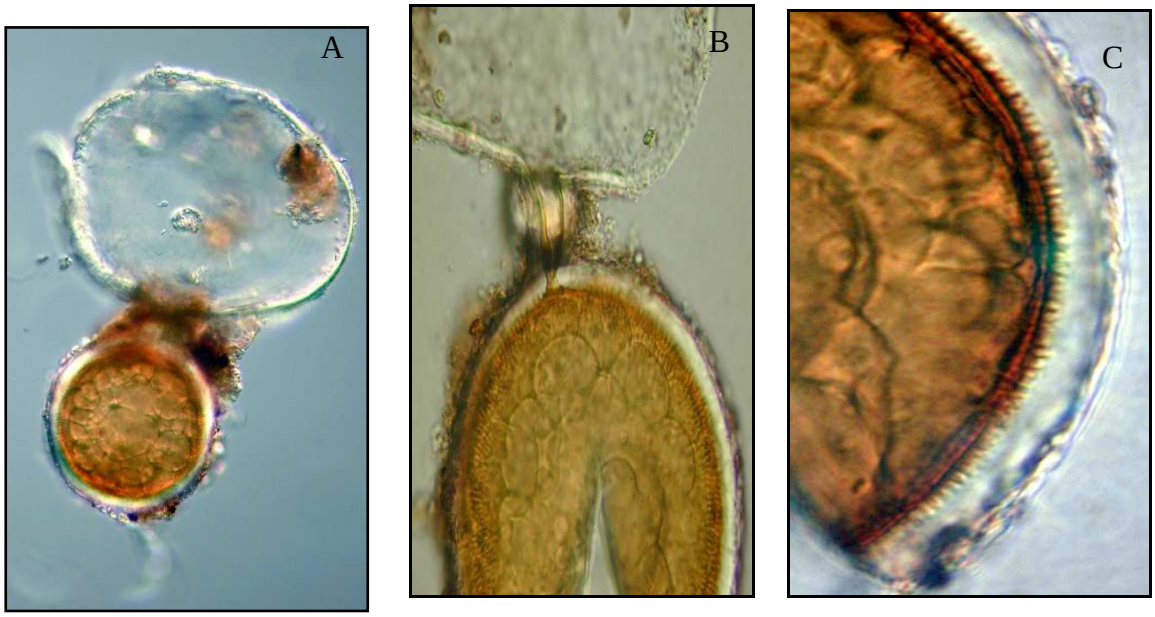

FIGURE 2: ENTROPHOSPORA INFREQUENS. A.YANG SPORE AND VESICLE, B AND C. VACUOLATED PROJECTIONS OF INNER SPORE WALL AND CLEAR OUTER MEMBRANE

A difference is marked in the soils of the site OG, never having door of culture of sugar beet, the mycorrhizal flora is diversified with representatives of several kinds of Glomeromycetes(*Acaulospora, Entrophospora, Gigaspora, Glomus, Pacispora and Scutellospora*), whereas the flora observed in the other sites with rotation mycotrophic plant and non-mycotrophic, contains predominantly of spores of the genus Glomus. We also have reported the abundance spore population of *P. scintillans* "Figure 1", which is considered as a record for the North Africa, this species is common to all regions with the exception of GH 2, this last has proved the least rich in diversity, in which only 2 species have been found are *G. mosseae, G.macrocarpum*"Table4".We also note the presence of interesting endophytic Entrophospora infrequens "fig 2". The researches on this endophytic are very important, it synthesize the anticancer alkaloid camptothecin (Amna et al., 2006, Puri et al., 2007).

IV. DISCUSSION AND CONCLUSION

In light of these results, it clearly appears that the cultivation of sugar beet, which is a non mycotrophic plant and is widely cultivated in the Doukkala region, acts negatively on the rate of spores of mycorrhizal fungi and this was confirmed in the two regions. A similar result was found by Black and Tinker (1979) on the rotation Barley- Beet.Number of spores quantified showed dominance spores whose size is between 50-100µm in both regions, indicating these fungi are preferred by this type of rotation

Although there is no host specificity in arbuscular mycorrhizal symbiosis, these results show the existence of an ecological specificity consistent with results of Gollotte et al. (2002) in prairie soils. Similarly, Oehl and al. (2003) showed that agricultural intensification causes a significant reduction in species diversity of arbuscular mycorrhizae. This leads us to assume that the cereal-beet rotation practiced over many successive years in the study area or the conduct of monocultures, will probably lead to a decrease in species diversity. In both regions, the analysis of the roots of cereals grown after beet showed a reduction in the amount of arbuscules, indicating a disturbance in the relationship between the two partners and affects the physiology and thus the growth of the plant. This similar behavior in the two regions shows that the plant reacts in the same way.

Concerning the biodiversity our results are consistent with other studies which have demonstrated the predominance of species of the genus *Glomus* in arid and semi-arid areas that adapt to water stress and saline.The *Glomus* species of were often cited as abundant species in the cultivated soil (Kachkouch et al., 2012, Sghir et al., 2015). Other authors also reported the presence of Glomus in arable soils, is one of the reasons for their dominance and their adaptation to disturbed environment (Bakkali Yakhle et al., 2011).

The results suggest that the intensive culture of species non-mycotrophic such that the beet associated to other factors (physico-chemical, work the ground etc.) significantly reduces the diversity of arbuscular fungi which is reflected on the total number of spores, on diversity and on the mycorrhizal activity (Zhao and Zhao, 2007, Maurer et al., 2014). Although there is not a specificity host-fungus in the arbuscular symbiosis, the existence of an ecological specificity as highlighted by Gollotte et al. (2002) in grassland soils expressed in our case through the variations of the biodiversity of the mycorrhizal profile.

The results obtained in this work have shown the impact of the culture of a species not mycotrophic on the number of spores of mycorrhizal fungi, their diversity and on the potential of functioning of the mycorrhizae within the roots. In the light of these results, we can propose certain practices in order to preserve the strains mycorrhizae who inhabit our soils:

- Alternate lines of mycotrophic species such as leeks with beets. for maintaining mycorrhizal strains in soil.
- Avoid making the same rotations on the same plots, which may favor certain species of mycorrhizal fungi and thus reduce diversity.
- Avoid rotation (beet- beet) which leads to further reduce mycorrhizal population..

A rational management of our agricultural soils would keep this fundamental heritage necessary for sustainable agriculture.

ACKNOWLEDGEMENTS

We thank the Regional Council of Doukkala to finance this work

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