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ARBUSCULAR MYCORRHIZAL ASSOCIATION IN TOMATO IN EIGHT MICROAGROSYSTEMS, NORTHEASTERN ALGERIA

ZIANE Hana[1]*, KSENTINI Hana[1] and MEDDAD-HAMZA Amel[1]

1. Badji Mokhtar Annaba University , Facukty of Sciences, Department of Biology, Algeria.

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Abstract

Description of the subject : Arbuscular mycorrhizal fungi (AMF) are telluric microorganisms which mediate interactions between plants and allow them to exploit the resources of the soil and a better tolerance to biotic and abiotic stress ; thus, they are fundamental to plant growth and development. However, in agricultural systems, the local AMF communities can be altered by conventionnal practices, and in the long term, their maintenance in agricultural soils can be compromised.
Objective : The aim of this study was to prospect the mycorhizal status of tomato crop in conventionnally managed agrosystems through the assessment of AMF root colonization levels and sporal density in soil.
Methodes : For this, eight agricultural soils in the northeast region of Algeria were chosen for estimating AMF root colonization parameters such as frequency (F%) and intensities of colonization (m% and M%) ; spores were isolated and quantified in 100 g of soil.
Results : The results showed that AMF colonization was established in tomato roots in all the studied sites with F% averaging 95.67% ; however, m% and M% were low to medium and ranged from 10.74% to 49.25%. Spore density was relatively low and ranged from 57 to 201 per 100 g of soil.
Conclusion : The results put forward the hypothesis that conventional management of soils in this region affects negatively the indigenous AMF population associated with tomato crop. This effect on probably hindering the expression of their beneficial effect, these sites might require exogenous AMF inputs as biofertilizers and biofortifiers for a more eco friendly agriculture.
Keywords : Agrosystems ; Mycorrhizae ; Root colonization ; *Solanum lycopersicum* (L.) ; Northeast Algeria.

ASSOCIATION MYCORRHIZIENNE ARBUSCULAIRE CHEZ LA TOMATE DANS HUIT MICRO-AGROSYSTÈMES, NORD-EST DE L’ALGÉRIE

Résumé

Description du sujet : Les champignons mycorhiziens à arbuscules (CMA) sont des micro-organismes telluriques médiateurs des interactions entre les plantes et leur permettent d'exploiter les ressources du sol et une meilleure tolérance aux stress biotiques et abiotiques ; ainsi, ils sont fondamentaux pour la croissance et le développement des plantes. Cependant, dans les systèmes agricoles, les communautés locales de CMA peuvent être altérées par les pratiques conventionnelles, et à long terme, leur maintien dans les sols agricoles peut être compromis.
Objectifs : L'objectif de cette étude était de prospecter le statut mycorhizien de la culture de tomate dans des agrosystèmes gérés de manière conventionnelle à travers l'évaluation des niveaux de colonisation des racines par les CMA et de la densité des spores dans le sol.
Méthodes : Pour cela, huit sols agricoles de la région nord-est de l'Algérie ont été choisis pour estimer les paramètres de colonisation racinaire des CMA tels que la fréquence (F%) et les intensités de colonisation (m% et M%) ; les spores ont été isolées et quantifiées dans 100 g de sol
Résultats : Les résultats ont montré que la colonisation par les CMA était établie dans les racines de tomate dans tous les sites étudiés avec un F% moyennant 95,67 % ; cependant, m% et M% étaient faibles à moyens et variaient de 10,74% à 49,25%. La densité de spores était relativement faible et variait de 57 à 201 par 100 g de sol.
Conclusion : Les résultats ont émis l'hypothèse que la gestion conventionnelle des sols dans cette région affecte négativement la population indigène de CMA associée à la culture de la tomate. Cet effet entravant probablement l'expression de leur effet bénéfique, ces sites pourraient nécessiter des apports exogènes de CMA comme biofertilisants et biofortifiants pour une agriculture plus respectueuse de l’environnement.
Mots clés : Agrosystèmes ; Mycorhizes ; Colonisation racinaire ; *Solanum lycopersicum* (L.) ; Nord-est algérien.

* Auteur correspondant : Ziane Hana, E-mail: hana.ziane@hotmail.fr

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are soil microorganisms that form a mutual symbiosis with most of terrestrial plants [1]. AMF are mainly responsible for mineral and water nutrition and induce the resistance of plants against certain pathogens and abiotic stresses [2]. AMF are biofertilizers that enhance crop growth and production [3] and improve soil aggregation and structure, which are fundamental properties of agricultural and natural ecosystems [4]. AMF are essential for the functioning of ecosystems, they induce fluctuations in grasslands like plant biodiversity, productivity and variability; when AMF diversity is poor, changes in the plant species composition and total structure of microcosms which dynamize grasslands may occur. On the contrary, an increased AMF richness can augment significantly plant biodiversity, nutrient acquisition and productivity [5].

Changes in the environment show that the use of chemical fertilizers and synthetic pesticides has reached its limits: pollution and loss of biodiversity in regions of intensive agriculture, degradation of systems more fragile agricultural products, prohibitive costs for the poorest producers [2]. Conventional farming practices influence considerably the persistence and development of AMF in soils. Mycorrhizal structures like spores and hyphae may be exposed to disturbances; thus, local AMF communities are periodically tested by the rapid renewal of host plants, crop rotations and soil exploitation, in particular in annual crops [6]. These practices will thereby negatively affect AMF communities in their diversity and their potentiality to colonize the roots of cultures [7, 8].

In Algeria, tomato (*Solanum lycopersicum* L.) occupies an area of 24,000 ha and produced 536,467 t/ha in 2017 [9]. This crop has adapted to a wide variety of climatic conditions and grows best in soils with adequate and sufficient nutrient supply [10]. Tomato has also been demonstrated to be greatly dependent on arbuscular mycorrhizae [11]. Therefore, the objective of this work was to prospect tomato root colonization by AMF and sporal density in eight micro-agroecosystems in northeast Algeria, where conventional agricultural practices are followed.

MATERIAL AND METHODS

1. Study sites

Tomato root colonization by AMF and their diversity were assessed in eight fields from north-eastern Algeria (provinces of Annaba, El Tarf and Guelma). Site 1 was situated in El-Karma locality, site 2 in Ben Amar, sites 3, 4 and 5 in Chbaïta Mokhtar, site 6 in Drean, site 7 in Denden, and site 8 in Djebel Houara locality. This region is characterized by a sub-humid climate, rainfall is higher in winter than in summer, the hottest month of the year is August and the coldest is January. The coordinates, annual rainfall and annual average temperatures of the sites are mentioned in table I. The farmers at these sites follow the technical sheets of the Technical Institute of Market Gardening and Industrial Crops, which include tillage, fertilization which consists of adding a basic fertilizer: organic (30 to 35 t/ha) and mineral (130 units of N/ha 120 units of P/ha 150 units of K/ha), maintenance manure: in 2 inputs 1st report one month after planting: (3 qx of N i.e. 100 units/ha) and 2nd input (1 ql of N i.e. 15 units/ha 1.5 to 2 qx of K i.e. 50 units / ha). For phytosanitary protection, Mancozebe is used against mildew and Chlorothalonil sc against whiteflies.

Table 1: Coordinates, annual rainfall (mm) and average annual temperature (°C) of study sites in the northeast region of Algeria

Study sites	Coordinates	Annual rainfall (mm)	Average annual temperature (°C)
Site 1 (El-Karma, Annaba)	36° 44’ 35’’N ; 7° 39’ 59’’E	699	18.2
Site 2 (Ben Amar, El-Tarf)	36° 46’ 55’’N ; 7° 48’ 35’’E	690	18
Site 3 (Chbaïta Mokhtar, El-Tarf)	36° 77’ 09’’N ; 7° 78’ 03’’E	705	18.1
Site 4 (Chbaïta Mokhtar, El-Tarf)	36° 75’ 24’’N ; 7° 72’ 97’’E	705	18.1
Site 5 (Chbaïta Mokhtar, El-Tarf)	36° 75’ 26’’N ; 7° 72’ 99’’E	705	18.1
Site 6 (Denden, El-Tarf)	36° 73’ 15’’ N ; 7° 78’ 38’’E	692	18
Site 7 (Drean, El-Tarf)	36° 69’ 49’’N ; 7° 73’ 63’’E	684	18
Site 8 (Djebel Houara, Guelma)	36° 59’ 20’’N ; 7° 61’ 82’’E	557	17.2

1. Root and soil sampling

In each tomato field, roots and rhizospheric soil were sampled in July 2017. Five plants were chosen randomly in each site, thin roots were harvested in the first 30 cm of the soil. Roots of each plant was sampled at least at three points which were subsequently mixed to form a single representative sample of the plant. Roots were then used for estimating colonization. Rhizospheric soil associated with tomato was collected from three points in each site (500 g) in the first 30 cm of the soil, then samples were homogenized to give a single representative sample. Soils were stored at 4° C for spore isolation and counting.

3. Estimation of root colonization levels

The roots were stained following Phillips and Hayman's technique [12]. Fresh roots were rinsed then placed in a 10% KOH solution at 90 °C for 20 min, then rinsed under running water. Finally, they were covered with a solution of trypan blue (0.05%) at 120 °C for 20 minutes and then rinsed with distilled water. The observations were made on 5 repetitions of 30 root fragments of 1 cm, placed between slide and coverslip in a drop of glycerol. The annotation was made according to a class scale to estimate the degree of mycorrhizal colonization according to the method described by Trouvelot et *al.* [13], using the software MYCOCALC (www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html). Three root colonization parameters were calculated:

-F % is the frequency of mycorrhizal colonization and represents the% of the number of mycorrhizal root fragments, it reflects the importance of the penetration points of the colonization of the root system.

-M % is the intensity of colonization of the root cortex, this is the proportion of the colonized cortex estimated in relation to the entire root system and expressed in %, it reflects the importance of colonization of the root system.

-m % is the intensity of colonization developed in the mycorrhized part of the root system, it is the proportion of the colonized cortex in the mycorrhized part of the root system expressed in%.

4. Isolation and counting of AMF spores

The spores were extracted by wet sieving by placing 100 g of soil on top of a series of sieves stacked from top to bottom according to the decreasing value of their mesh size (300, 250, 200, 150, 100 and 50µm). The soil was then thoroughly washed until the flowing water became clear. Each soil fraction retained in each sieve was collected and observed under a binocular magnifying glass [14]. The total number of healthy spores was counted per 100 g of soil, repeated three times.

5. Statistical analysis

A single factor ANOVA was performed for root colonization levels and spore density. Segregation between means was made with the Tukey test Honestly Significant Difference (HSD) at the 5% level. Statistical analysis were carried out with R statistical program version 4.0.4.

RESULTS

1. Root colonization levels

Estimation of AMF root colonization of tomato in the eight fields revealed that colonization occurred in all tomato roots; however, their colonization levels varied from one site to another. Mycorrhizal frequency (F%) was high in all the sites and ranged from 85.08% to 98.82%, with an average of 95.67% (Fig. 1).

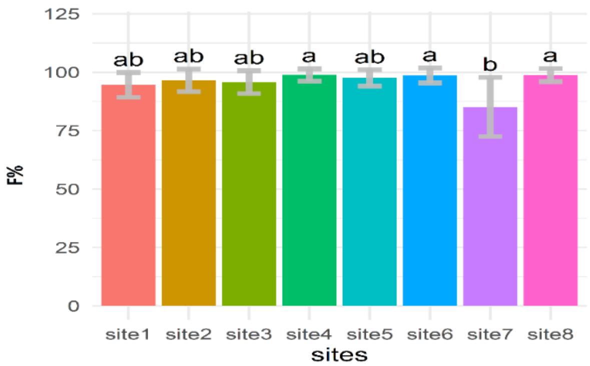

Figure 1: Frequency of mycorrhizal colonization (F%) of tomato plants in eight fields in northeastern Algeria.

Intensity of mycorrhization in the entire cortex (M%) was relatively low to medium, with an average of 29.66%. The highest value was present in sites 4 and 5 (48.25% and 49.25%, respectively) and the lowest occurred in site 7 (10.74%) (Fig. 2). Intensity of colonization in the mycorrhizal part of root system (m%) was relatively low to medium and ranged from 10.97% to 49.25%, with an average of 30.28% (fig. 3).

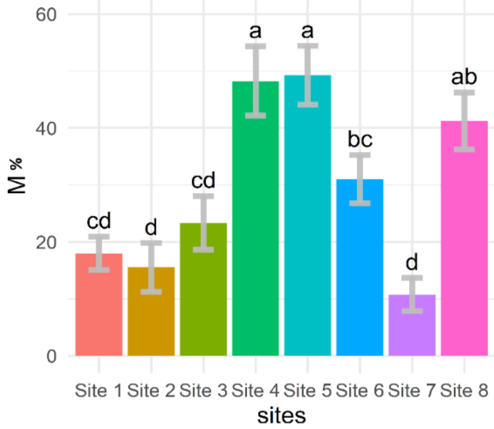

Figure 2: Intensity of colonization of the root cortex (M%) of tomato plants in eight fields in northeastern Algeria.

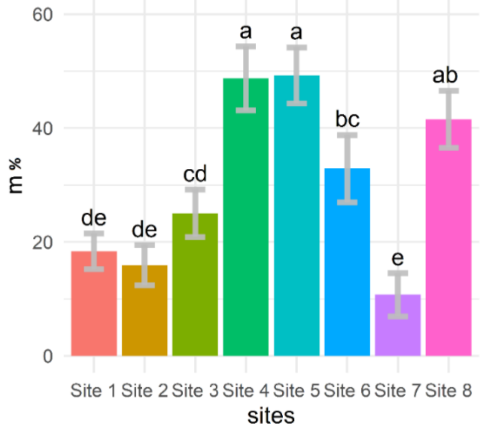

Figure 3: Intensity of colonization in the mycorrhized part of the root system (m%) of tomato plants in eight fields in northeastern Algeria.

2. AMF spore density

AMF spore density in 100 g of rhizospheric soil associated with tomato was relatively low, with an average of 135.75±15.87. The highest spore density was observed in site 4 (201±17 spores 100 g^{-1}) and the lowest was in site 7 (57±12 spores 100 g^{-1}) (Fig. 4).

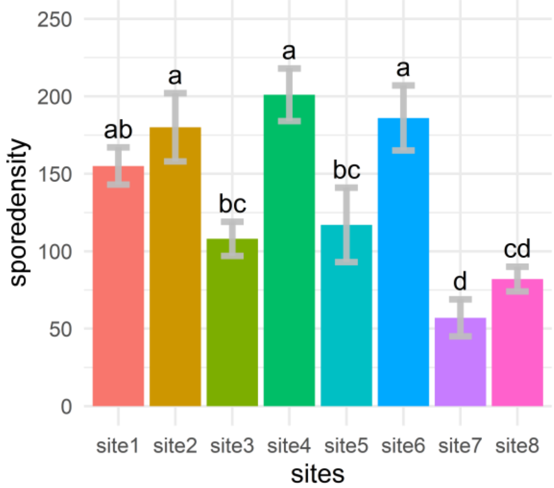

Figure 4: AMF spore density (spores 100 g^{-1}) in eight tomato fields in northeastern Algeria

DISCUSSION

This study contributed to provide information on arbuscular mycorrhizal association in tomato, by assessing AMF colonization in roots and sporal density in conventionally managed soils from northeastern Algeria. AMF are obligate symbionts that necessitate a host plant to achieve their life cycle [15], they infect roots using propagules in the soil such as spores. However, agricultural practices can cause disturbances that impact negatively both dynamics and diversity of AMF, lowering the colonization levels in roots and propagules density in the soil.

In this work, intensity of colonization by AMF in the eight fields had an average of 29.66%, which is higher compared to the results obtained by Cavagnaro & Martin [16] in 26 micro-agroecosystems where tomato roots were colonized at less than 15% and colonization was completely absent in more than 40% of the soils.

Sites 4 and 5 had the highest intensity of colonization compared with other sites, which can be explained by the influence of soil physical and chemical properties such as P and organic matter rates, aeration, pH, texture, crop management on AMF infection in roots and seasonality. Low levels of mycorrhizal colonization can be attributed to the prejudicial impact of chemical fertilizers, pesticides and tillage, which lower AMF growth and root colonization [16, 17].

Hage-Ahmed *et al.* [6], reported that during the pre-symbiotic phase of AMF, pesticides can prevent the establishment of the symbiosis by having a negative effect on spore germination and mycelium growth. The spores can remain without germinating after being exposed to pesticides and thus, can germinate and colonize the host plant after the reducing, metabolism or even degradation of the inhibitory active substances. When AMF germinate and colonize the host plant, if the mycelium and the spores are affected by active substances, a reduction in the symbiosis and thus the transport of P to the plant might happen [6].

In this work, AMF sporal desnities in soils cultivated with tomato (135.75 in 100g) was low compared to those previously found in a conventional tomato field in the Mediterranean region (395.5 in 100g) [18]. Oehl *et al.* [19], demonstrated in a comparison study between organic and conventional farming that the number of AMF spores was significantly reduced by conventional cultivation practices. This can be explained by the detrimental effects of pesticides application [20], chemical fertilization [21] and tillage, which has a negative impact on AMF by the destruction of their extra-radical hyphae networks [17, 22].

Presently, the interest on soil biodiversity is expanding in view of their potential as substitute or advantageous biotechnologies to synthetic agricultural products. Crop yields can be improved by AMF based inocula, as they give several services to crops [23].

CONCLUSION

Tomato crop was associated with AMF in northeastern Algeria. However, root colonization levels by AMF and sporal density were relatively low at most sites. These results taken together show that conventional management of soils is detrimental to local AMF communities. The potential benefits attributed to AMF are probably no longer fully utilized and therefore need to be restored in the most disturbed agroecosystems.

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