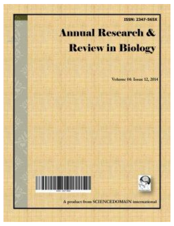

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Study of the Diversity of Arbuscular Mycorrhizal Fungi in the Highway Slopes of Taza Region (North-East of Morocco)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The diversity of arbuscular mycorrhizal fungi (AMF) was examined in the cut and filled slopes of Taza region (North-East of Morocco). The identification of the spores isolated from these two types of slopes has revealed 29 species belonging to 7 genera: *Glomus* (14 species), *Acaulospora* (6 species), *Gigaspora* (2 species), *Pacispora* (3 species), *Entrophospora* (2 species), *Scutellospora* (1 species), *Funelliformis* (1 species), 6 families (*Glomeraceae, Gigasporaceae, Acaulosporaceae, Pacisporaceae, Scutellosporaceae, Entrophosporaceae*) and 3 orders (Glomerales, Gigasporales, Diversisporales). The number of specific species to cut and filled slopes is respectively 12 and 6 species and the number of species common to the two types of slopes is 11 species. All these species will serve, after multiplication, as sources of endomycorrhizal inoculum, which can enhance the growth and increase the resistance of plants, used for vegetation and restoration of the slopes.

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1. INTRODUCTION

The construction of roads and highways in different regions of Morocco has resulted in the creation of numerous slopes, often predisposed to erosion and slippage due to several environmental factors (climate, soil type, topography, vegetation cover) [1,2]. The instability of slopes and natural embankments remains a problem especially in relief areas, in view of the problems of infrastructure maintenance and risk management. Chehlafi et al. [3] have shown that water erosion is one of the problems encountered in road construction. Studies carried out in this context have shown that slopes and natural embankment without vegetation have an impact on soil erosion. Thus, slope vegetation is necessary to limit erosion and reduce the risk of instability and subsequently to protect the road network.

This technique needs to be improved and enhanced by other biotechnological techniques based on the trapping and multiplication of useful microorganisms. This is the case of endomycorrhizal fungal species which are capable of stabilizing soil aggregates through the release of glomalin promoting the establishment and development of plants under difficult conditions [4].

Recent studies [5] have shown that mycorrhizae play an important role in maintaining plants in their natural biotopes and regeneration. However, mycorrhizal plants have never been used in Morocco for slope vegetation. For this reason, in order to evaluate mycorrhization, the present work consists in studying the diversity of arbuscular endomycorrhizal fungi at the cut and filled slopes of Taza region (North-East of Morocco).

2. MATERIALS AND METHODS

2.1 Sites of Samplings

Soil samples were collected along the highway linking Fez to Taza at a depth of 20 cm, from two sites (Tahla (Fig. 1A) and Oued Amlil (Fig. 1B)). Sampling of the soil samples was carried out in July-August, at a depth of 0 to 20 cm. For each site, 5 random samples (2 kg of soil per sampling) were collected from made in each slope and a composite soil sample was produced per site. No sampling was carried out in the rhizosphere of indigenous plant species.

2.2 Spores Extraction

The spores were extracted according to the wet sieving method described by Gerdemann and Nicolson [6]. In a 1L beaker, 100 g of each composite soil sample was submerged in 0.5 L of running water and stirred for 1 min with a spatula. After 10 to 30 seconds of

Fig. 1. Cut slope of Tahla (A), filled slope of Oued Amlil (B)

decantation, the supernatant was passed through four superposed sieves with decreasing meshes (500, 200, 80 and 50 µm), this operation was repeated twice. The content retained by the 200, 80 and 50 µm sieves was distributed in two tubes and centrifuged for 4 min at 9000 rpm. The supernatant was discarded and a viscosity gradient was thus created by adding 20 mL of a 40% sucrose solution to each centrifuge tube [7]. The mixture was rapidly stirred and the tube was returned again to the centrifuge for 1 min at 9000 rpm.

Unlike the first centrifugation step, the supernatant was poured onto the sieve with a mesh of 50 microns; the substrate obtained was rinsed with distilled water to remove sucrose and then disinfected with an antibiotic solution (Streptomycin). The spores were then recovered with a little distilled water in an Erlenmeyer flask.

Spores were observed under an optical microscope and identified morphologically based on spore color, shape, size, surface ornamentation, spore contents and wall structures, sporulous saccule, germination shield, bulb and suspensor [8,9,10]. Slides of each different spore morphotype were prepared using either polyvinyl-alcohol alone or mixed with Melzer's solution [9,11]. The identification of spores was made according to species descriptions provided by the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM) [12] following the classification of Redecker et al. [13].

Table 1. Identification of endomycorrhizal fungi isolated from the cut slopes (Oued Amlil)

Number	Species	Form	Color	Spore's size (µm)	Wall's Size (µm)	Spore's surface	Hyphae length (µm)
1	*G. intraradices*	Oval	Yellow	70	2.5	Smooth	112.5
2	*G. macrocarpum*	Oval	Orange	75	7.5	Smooth	-
3	*Gigaspora* sp1	Globular	Yellow	82.5	2.5	Granular	-
4	*A. laevis*	Globular	Yellow Brown	70	2.5	Granular	-
5	*Acaulospora.* sp1	Globular	Yellow	75	2.5	Granular	-
6	*G.geosporum*	Globular	Yellow Brown	70	2.5	Smooth	-
7	*A. colossica*	Globular	Brown dark	62.5	2,5	Smooth	-
8	*Gi.margarita*	Globular	Yellow	57.5	2.5	Smooth	-
9	*G .aggregatum*	Globular	Brown Yellow	52.5	2.5	Smooth	52.5
10	*Glomus*.sp1	Globular	Orange	85	2.5	Granular	-
11	*Glomus* sp2	Oval	Orange clear	57.5	2.25	Granular	-
12	*G .etunicatum*	Globular	Yellow Brown	62.5	5	Smooth	-
13	*A. gedanensis*	Globular	White Yellow	87.5	2.5	Smooth	-
14	*Acaulospora.* sp2	Globular	Yellow clear	26	2.5	Granular	-
15	*P. robiginia*	Globular	Green clear	37	2.5	Smooth	-
16	*G.versiforme*	Globular	Yellow Brown	38	2.5	Granular	-
17	*G .clarum*	Globular	Brown	62.5	2.5	Granular	-
18	*P .scintillans*	Globular	Brown white	45	2.5	Granular	-
19	*F. mosseae*	Oval	Yellow green	26	2.5	Granular	-

A: Acaulospora, F: Funneliformis, E: Entrophospora, G: Glomus, P: Pacispora, S: Scutellospora, Gi: Gigaspora

Table 2. Identification of endomycorrhizal fungi isolated from the cut slopes studied (Tahla)

Number	Name	Form	Color	Spore's size (µm)	Wall's size (µm)	Spore's surface	Hyphae length (µm)
1	*A. gedanensis*	Oval	White yellow	36	35	Smooth	5
2	*A. denticulata*	Oval	Orange	38	15	Granular	-
3	*G.versiforme*	Globular	Brown darkened	29	2.5	Granular	-
4	*Glomus* sp1	Globular	Brown white	27	2.5	Smooth	5
5	*A .leavis*	Globular	Yellow brown	30	5	Smooth	-
6	*A. colossica*	Globular	Yellow white brown	33	2.5	Smooth	-
7	*Acaulospora* sp1	Globular	Yellow	40	2.5	Smooth	-
8	*Acaulospora* sp 2	Globular	Orange white	34	2.5	Granular	-
9	*G .glomerulatum*	Globular	Brown darkened	30	2.5	Smooth	12
10	*G.trimurales*	Globular	Brown darkened	29	2.5	Granular	-
11	*G.clarum*	Globular	Yellow brown	52.5	2.5	Granular	
12	*G. macrocapum*	Globular	Yellow	40	2.5	Granular	-
13	*Enterosphospora* sp.	Globular	Yellow brown	1.9	2.5	Granular	-
14	*G .deserticola*	Globular	Orange	5.4	2.5	Smooth	-
15	*G. geosporum*	Globular	Brown darkened	175	2.5	Smooth	-
16	*S. heterogama*	Globular	Yellow darkened	38	3.33	Granular	-

A: Acaulospora, F: Funneliformis, E: Entrophospora, G: Glomus, P: Pacispora, S: Scutellospora, Gi: Gigaspora

Table 3. Identification of endomycorrhizal fungi isolated from filled slopes (Oued Amlil)

Number	Species	Form	Color	Spore's size (µm)	Wall's Size (µm)	Spore's surface	Hyphae length (µm)
1	*G. intraradices*	Globular	Yellow	38	2.5	Smooth	-
2	*A .denticulata*	Globular	Orange	30	2.5	Smooth	-
3	*Glomus* sp1	Globular	Orange	39	2.5	Smooth	-
4	*G .versiform*	Oval	yellow brown	37	3.5	Granular	-
5	*S.heterogamma*	Globular	Orange	30	3.5	Granular	-
6	*G .clarum*	Globular	Yellow	23	3.5	Granular	10
7	*Acaulospora* sp1	Globular	Yellow white	62.5	2.5	Granular	-
8	*A .colossica*	Globular	Clear green	42	2.5	Granular	-
9	*P. dominika*	Globular	Yellow white	112.5	2.5	Granular	-
10	*Acaulospora leavis*	Globular	Brown clear	31	2.5	Granular	-

A: Aculospora, F: funneliformis, E: Entrophospora, G: Glomus, P: Pacispora, S: Scutellospora, Gi: Gigaspora

Table 4. Identification of endomycorrhizal fungi isolated from the filled slope of Tahla

Number	Species	Form	Color	Spore's size (µm)	Wall's Size (µm)	Spore's surface	Hyphae length (µm)
1	*G.geosporum*	Globular	Brown	62.5	2.5	Smooth	-
2	*G.melanosporus*	Globular	Yellow	102.55	2.5	Granular	-
3	*G .clarum*	Globular	Yellow darkened	97.5	3.33	Granular	-
4	*Acaulospora* sp1	Globular	Yellow orange	90	2.5	Smooth	-
5	*G .étunicatum*	Globular	Orange	105	2.5	Granular	-
6	*G. intraradices*	Globular	Yellow	50	2.5	Smooth	-
7	*G .aggregatum*	Oval	Yellow	75	2.5	Granular	-
8	*G .versiforme*	Globular	Yellow	75	2.5	Granular	-
9	*G. gigantea*	Oval	White brown	32	2.5	Smooth	-
10	*E. inferquens*	Globular	Brown darkened	28	2.5	Granular	-
11	*Acaulospora* sp 2	Oval	Yellow	25	2.5	Granular	-

A: Aculospora, F: Funneliformis, E: Entrophospora, G: Glomus, P: Pacispora, S: Scutellospora, Gi: Gigaspora

Table 5. Relative abundance of endomycorrhizae in different study areas

Species	Spore's number/100 g of soil			
	Oued Amlil region		Tahla region	
	Cut slope	Filled slope	Cut slope	Filled slope
A. laevis	2	2	1	-
A. colossica	1	1	1	-
A.denticulata	-	1	2	-
A. gedanensis	1	-	1	-
Acaulospora sp 1	3	-	-	1
Acaulospora sp 2	.	1	3	1
Entrophospora sp	-	-	1	-
E. inferquens	-	-	-	2
F. mosseae	1	-	-	-
Gi. margarita	1	-	-	-
Gigaspora sp1	2	-	-	-
G. etunicatum	1	-	-	1
G. glomerulatum	-	-	1	-
G. intraradices	5	3	-	2
G. versiforme	4	1	6	2
G. macrocarpum	1	-	2	-
G.melanosporus	-	-	-	2
G.geosporum	1	-	1	1
G. clarum	1	2	7	3
G. aggregatum	3	-	-	1
G. trimurales	-	-	1	-
G. gigantia	-	-	-	1
G.deserticola	-	-	1	-
Glomus sp 1	2	1	-	-
Glomus sp 2		-	1	-
P. scintillans	1	-	-	-
P. robiginia	1	-	-	-
P. dominika	-	1	-	-
S. heterogama	-	1	1	-
Total of spores	31	14	30	17

A: Aculospora, F: Funneliformis, E: Entrophospora, G: Glomus, P: Pacispora, S: Scutellospora, Gi: Gigaspora

2.3 Species Richness and Frequency of Occurrence of Spores

Species richness is the total number of species observed in every sampling site and the frequency of occurrence of species corresponds to the percentage of sites where each species is detected.

2.4 Statistical Analysis

The statistical treatment of results focused on the analysis of variance with a single classification criterion (ANOVA1).

3. RESULTS

According to this study, 29 species of mycorrhizal fungi have been identified: *Acaulospora laevis, A. colossica*, *A. denticulate*, *A. gedanensis*, *Acaulospora* sp1, *Acaulospora* sp2, *Entrophospora* sp., *Entrophospora inferquens*, *Funneliformis mosseae*, *Gigaspora margarita*, *Gigaspora* sp1, *Gigaspora gigantean, Glomus etunicatum*, *G. glomerulatum*, *G. intraradices*, *G. versiforme*, *G. macrocarpum*, *G. melanosporus, G. geosporum*, *G. clarum*, *G. aggregatum*, *G. trimurales*, *G. deserticola*, *Glomus* sp1 , *Glomus* sp2, *Pacispora scintillans*, *P. robiginia*, *Pacispora dominika*, *Scutellospora heterogama*.

According to the classification of Schenck and Pérez [9], Goto [10], Oehl et al. [14] the species are divided into 7 genera: *Glomus, Acaulospora, Gigaspora, Pacispora, Entrophospora, Scutellospora* and *Funelliformis*; 6 families: (*Glomeraceae, Gigasporaceae,* and

Acaulosporaceae, Pacisporaceae, Scutellosporaceae, Entrophosporaceae) and 3 orders (Glomerales, Gigasporales, Diversisporales). The fungi isolated from the rhizosphere of different sites (Oued Amlil and Tahla) and from each slope (filled and cut slopes) were identified according to their morphology (Tables 1, 2, 3 and 4), indeed, the number of endomycorrhizal species isolated at the cut slopes of Oued Amlil and Tahla, 17 and 15 species respectively was greater than that recorded at the filled slopes studied, 10 species, in each region.

At the cut slopes in the Oued Amlil region, *Glomus intraradices* and *Glomus versiforme* were the most abundant with 5 and 4 spores / 100g of soil respectively. However, for the Tahla region, the most abundant species were *Glomus clarum* and *Glomus versiforme*, with respectively 7 and 6 spores / 100 g of soil and a co-dominance between the other species (Table 5).

In this preliminary study, the dominant species in all the sites studied is *Glomus versiforme* with a percentage of 14.89%, followed by *Glomus clarum* (13.82%) and *Glomus intraradices* (11.7%) (Fig. 2). It is thus observed that the genus *Glomus* is the most frequent (Fig. 3).

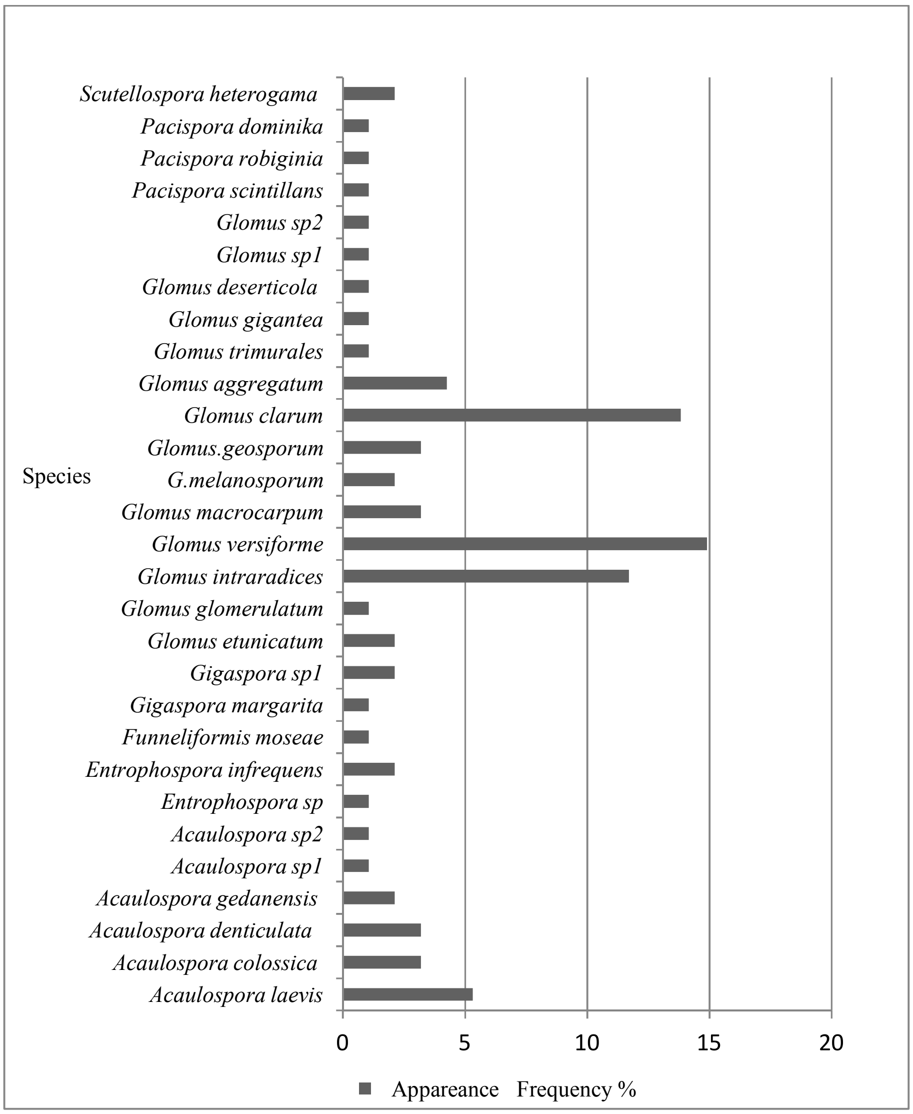

Fig. 2. Appearance frequency of species at different study sites

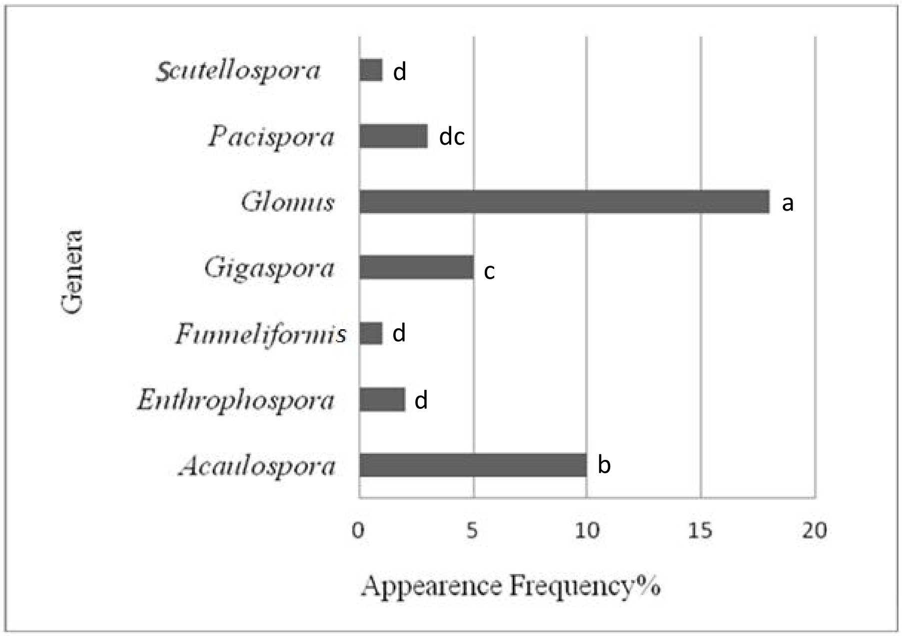

Fig. 3. Appearance frequency of genera at different study sites two results with the same letter do not differ significantly at the 5%

4. DISCUSSION AND CONCLUSION

The construction of different types of slopes has affected the diversity and abundance of mycorrhizal propagules and, as a consequence, reduced the mycorrhizogenic potential of soils. The results obtained from this work have shown that the soil disturbance by the building work has a negative effect on endomycorrhizal diversity in all types of slopes (filled and cut slopes). The identification of the spores isolated from the two slopes has revealed 29 species belonging to 7 genera: *Glomus* (14 species), *Acaulospora* (6 species), *Gigaspora* (2 species), *Pacispora* (3 species), *Entrophospora* (2 species), *Scutellospora*, *Funelliformis,* 6 families (*Glomeraceae, Gigasporaceae, Acaulosporaceae, Pacisporaceae, Scutellosporaceae, Entrophosporaceae*) and 3 orders (Glomerales, Gigasporales, Diversisporales). However, the filled slopes had a higher spore density than the cut ones.

The soil collected from the two studied slopes contains spores of a large number of endomycorrhizal fungi. This soil probably contains other species that have not been detected. The cultivation of different mycotrophic plant species on the soil of the two types of slope may probably favor the appearance of other endomycorrhizal fungal species not encountered during the first soil tests. The diversity of endomycorrhizal species may also be important if sampling is carried out, in different seasons, at the level of the rhizosphere of the autochthonous plant species present in the slopes.

In Morocco, dominance of the genus *Glomus* has been reported in different soil types and in the rhizosphere of several plant species, olive and oleaster [15,16,17], carob tree [18,19], date palm [20], *Argania spinosa* [21], *Citrus* [22] and *Sugar cane* [23].

Bouazza et al. [24] also noted the dominance of this genus in the coastal dunes of Tegra (northwestern Algeria) after a massive industrial exploitation of soil. This dominance was explained by the fact that the genus *Glomus* is the most suitable for environmental disturbances [25].

Representatives of the following genera *Acaulospora, Gigaspora, Pacispora, Entrophospora, Scutellospora,* and *Funelliformis* were also observed in the two types of slope studied but were however less dominant than those of the genus *Glomus*. Oehl et al. [26,27] have shown that crop intensification in agricultural soils also leads to a significant reduction in the diversity of endomycorrhizal spores. These authors also noted the impact of plowing on the mychorrizogenic potential of the soil. In fact, plowing limits the abundance of arbuscular mycorrhizal fungi in the upper soil layer. The same results have been reported by Diop et al. [28], which showed that the soils of disturbed ecosystems contain very few spores. Other studies have also shown that soil disturbance may affect the distribution of arbuscular mycorrhizal fungi [29]. All these results are consistent with those found in the studied slopes. Indeed, earthworks, loading and unloading, can cause degradation of endomycorrhizal spores and their disappearance.

In order to remedy the microbiological deficiencies due to the construction of the slopes, some strategies will be proposed, it is important to determine the mycorrhizal status of the plant species already developing or likely to develop in slopes, select the mycotrophic plant species for vegetation of slopes and to multiply a composite endomycorrhizal inoculum (several species) from the species isolated from the disturbed slopes.

These species will be selected according to their high infectivity on the plant species which will show an adaptation to the different climatic and edaphic conditions of the slope. The success of slope vegetation is dependent on the development of new techniques in nurseries for production of seedlings of a high-quality adaptable to different pedoclimatic conditions of the slopes, once they are re-planted.

In this sense, Touati et al. [30] studied the effects of inoculation with a composite inoculum containing several mycorrhizal species, of eleven species considered important for the restoration of slopes due to their common use at the slopes or by their existence in mobile dunes characterized by extreme ecological factors, namely, *Arundo donax, Spartium junceum, Atriplex halimus, Lavandula dentata, Medicago arborea, Coronilla emerus, Vetiveria nigritana, Chamaerops humilis, Retama monosperma, Lycium europaeum* and *Casuarina* sp.. The results obtained under greenhouse conditions showed that the inoculated plants were significantly better than the control ones in terms of growth and density of the aerial and root parts, except for *C. humilis* and *L. dentate* which showed poor performance results in terms of spore density, intensity and arbuscular content.

The results of this work are preliminary but will allow, over time, to improve knowledge on endomycorrhizal fungi and their use in revegetation sites. The fight against the instabilities and landslides remains a major environmental concern in all countries. Similarly, the integration of AMF in road slope revegetation programs should be an imperative step in all vegetation programs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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