Biodiversity Journal, 2014, 5 (4): 481–498

Little effect of ecological factors and symbiotic specificity on the distribution of *Medicago* subsect. *Intertextae* (Urban) Heyn (Fabales Fabaceae) in the Mediterranean Basin

Meriem Laouar^{*} & Aïssa Abdelguerfi

Ecole Nationale Supérieure Agronomique, Belfort, El Harrach 16200 Alger, Algérie; e-mail: laouar_m@yahoo.fr *Corresponding author

ABSTRACT

In the Mediterranean region, Medicago granadensis Willd. and M. muricoleptis Tineo (Fabales Fabaceae) of the Intertextae subsection are rare and present, each one in well defined areas, whereas the others taxa, in the same subsection, M. ciliaris (L.) All. and M. intertexta (L.) Mill., are more frequent with large distribution. This kind of geographical distribution is not a coincidence; certainly different factors are the origin of this distribution. In 125 sites of different Mediterranean countries, the ecology of the four taxa was studied, 34 trapped strains in one site of Intertextae rhizobiums were characterized by rep-PCR and some symbiotic tests were carried out. The results confirm that M. muricoleptis and M. granadensis are endemic in the north and east of the Mediterranean, respectively; whereas, M. ciliaris and M. intertexta are widespread. Although their geographic specificity, the four taxa showed no important differences in ecological conditions. M. muricoleptis, given its presence in the north of the Mediterranean only, is distinguished by its precipitation requirement. By inference, the absence of M. muricoleptis and M. granadensis in some regions is not due to the absence of the symbiont. We confirm the symbiotic specificity of Sinorhizobium medicae (Rome, 1996) for the Intertextae subsection and the existence of coevolution between taxa (widespread and endemic) and rhizobia. We suppose that endemic taxa are in the process of allopatric speciation, which explains their narrow distribution in the Mediterranean Basin.

KEY WORDS Distribution; diversity; ecology; *Intertextae*; *Medicago*; Mediterranean; symbiosis.

Received 15.10.2014; accepted 18.11.2014; printed 30.12.2014

INTRODUCTION

Legumes hold an important economic and social place and have a definite environmental benefit in the Mediterranean Basin, which is the area of distribution of several genera of this family, including the *Medicago* L. genus. This genus includes 86 species (Small & Jomphe, 1989) and comprises several sections and subsections, including the *Intertextae* (Urban) Heyn subsection (Small, 2011; Coulot & Rabaute, 2013). Some taxa of the *Inter*- *textae* show a morphological ambiguity, which reflected in different classifications. Indeed, some authors ascribe to the subsection 4 species, namely, *Medicago ciliaris* (L.) Krock., *M. intertexta* (L.) Mill., *M. muricoleptis* Tineo and *M. granadensis* Willd. (Quézel & Santa, 1962; Lesins & Lesins, 1979; Small & Jomphe, 1989; Coulot & Rabaute, 2013) and others mention only 3 species, *M. intertexta*, *M. muricoleptis* and *M. granadensis* (Heyn, 1963; Ponert, 1973; Jauzein, 1995; Dobignard & Chatelain, 2012; Tison et al., 2014). According to

the latter authors *M. ciliaris* is either a subspecies, *M. intertexta* subsp. *ciliaris* (L.), Ponert, or a botanical variety [*M. intertexta* var. *ciliaris* (L.) Camb, Heyn] of *M. intertexta*.

The early observations on the distribution of annual *Medicago* in relation with soil factors were made by Trumble & Donald (1938) and Aitken & Davidson (1954). Besides, the first work on autoecology was carried out in Australia by Andrew & Hely (1960). Thereafter, several studies were performed, particularly in the regions of origins of *Medicago*, which allowed the definition of the ecological requirements of various species (Abdelguerfi et al., 1988; Ehrman & Cocks, 1988; Prosperi et al., 1989; Ehrman & Cocks, 1990; Abdelkefi et al., 1992; Bounejmate, 1992; Bounejmate et al., 1992a; 1992b).

For the most common taxa of the Intertextae subsection, M. ciliaris and M. intertexta, autoecology research was carried out, but most of that research did not allow comparisons between the two taxa since they were included in a single species as indicated by Heyn (1963). The most specific study on these two taxa was carried out in Algeria (Abdelguerfi-Laouar et al., 2003) on 179 sites. Besides, M. muricoleptis and M. granadensis are poorly distributed and are reported in the literature as rare taxa. Although they belong to the same subsection as *M. ciliaris* and *M. intertexta*, they are not found in Algeria, Morocco, and Tunisia. The ecological requirements of the two taxa are not yet determined; they have only been reported in various regions (Table 1).

Other factors, besides ecological ones, symbiosis for example, may affect the distribution of legumes species. The species of rhizobia that nodulate the Medicago ssp. belong to the Ensifer (formerly Sinorhizobium) genus and are S. meliloti (Dangeart, 1926) and S. medicae (Rome, 1996). One of the major characteristics of the rhizobiumlegume association is their host specificity. In general, a given rhizobium species can only establish an efficient symbiotic relationship with a limited number of plant partners. Similarly, legumes species can only be specific to a certain number of rhizobium species. This coevolution is often ignored in studies on the distribution and evolution of legumes taxa. The Intertextae subsection taxa were described as specific to S. medicae (Béna et al., 1998; 2005). Béna et al. (1998) suggest, for this subsection, a recurrent loss of the capacity to form an efficient symbiosis with strains of the *S. meliloti* species; they hypothesize a punctual mutation inducing a modification of the mechanisms of recognition such as the flavonoid structure.

The rather particular geographical distribution of the four *Intertextae* subsection taxa, namely those with a wide distribution and those with a narrow distribution, needs clarification as to whether this is due to an ecological adaptation and/or to a specific taxon-rhizobium interactions; this is the objectives of this work.

MATERIAL AND METHODS

Given the taxonomic ambiguity that exists within the subsection of the *Intertextae*, we will use the term taxon instead of species for *M. ciliaris*; synonym: *M. intertexta* subsp. *ciliaris* and *M. intertexta* var. *ciliaris*, *M. intertexta*, *M. muricoleptis* and *M. granadensis* throughout the document.

Ecological data and origin of sites

The study is focused on taxa *M. ciliaris, M. intertexta, M. muricoleptis* and *M. granadensis*. The collected ecological data come from the Australian Medicago Genetic Resource Centre (South Australian Research and Development Institute: SARDI), a total number of 125 sites were surveyed in 17 Mediterranean countries and islands (Table 2). 13 sites of 125 are sympatric (sci and sig), one of which is sympatric to *M. intertexta* and *M. granadensis* (sig; Israel) and the other (12) are for *M. ciliaris* and *M. intertexta* (sci). All the other sites are allopatric (sc, si, sm and sg) and correspond only to a species per site among the four (Table 3).

Ecological data analysis

Nine (9) ecological characters were studied; they correspond to orographic (altitude and slope), climatic (annual rainfall) and edaphic characters (pH, soil texture, soil depth, soil reaction, type of drainage and land use). Analyses of variance (ANOVA) with a single factor (sites) were carried out on 76 sites whose data on rainfall, altitude, and pH were available; the 'site' factor was considered as random effect. The software used is STAT-ITCF

Species	Regions	References	
	- Macaronesia	Coulot & Rabaute 2013	
	Madeira Island (Portugal), Canary Island	Coulor & Rubuute, 2015	
	(Spain)	Davis, 1965-1988	
	- North Africa	Dobignard & Chatelain	
	Algeria, Egypt, Morocco, Tunisia	_ 2012	
	- Western Asia	2012	
M ciliania (I) All	Cyprus, Iraq, Israel, Lebanon, Western	Heyn, 1963	
M. churis (L.) All.	Syria, Western Turkey	Jahandiez & Maire 1931-1941	
	- South-Eastern Europe		
	Greece (including Crete), Italy (including	Lesins & Lesins, 1979	
	Sardinia and Sicily)	- Maikla 1077 1085	
	- South-Western Europe	Merkie, 1977-1985	
	France (including Corsica), Portugal and	Mouterde, 1966	
	Spain (including the Balearic Islands)	Pignatti 1082	
	- Macaronesia	- Tighaul, 1982	
	Canary Island (Spain)	Pottier-Alapetite, 1979-1981	
	- North Africa	- Ouézel & Santa 1962	
	Northern Algeria, Morocco, Tunisia	Quezer & Sunta, 1902	
<i>M. intertexta</i> (L.)	- South-Eastern Europe	Small & Jomphe, 1989	
Mill.	Greece, Italy (including Sardinia and	Small 1081	
	Sicily)	Small, 1981	
	- South-Western Europe	Small et al., 1981	
	France (including Corsica), Portugal and	Smythies 1084-1086	
	the South of Spain	Sinytines, 1964-1980	
	- North Africa	Täckholm, 1974	
M anavadansis	Northern Egypt	T 1.0.C 10//	
willd	- Western Asia	Townsend & Guest, 1966	
willd.	Israel, Lebanon, Jordan, Western Syria,	Tutin et al., 1964-1980	
	Turkey	,	
	- South-Eastern Europe	Zohary & Feinbrun-Dothan,	
	Southern Italy (including Sicily)	1966	
M. muricoleptis		GRIN 2014	
Tineo	- South-Western Europe		
	Southeast of the France near Toulon		

Table 1. Geographical distribution of the Intertextae subsection taxa.

(Gouet & Philippeau, 2002). For each quantitative variable, two analyses of variance were carried out; the first analysis comprised 4 levels corresponding to the presence of the taxon: (i) site of *M. ciliaris* (sc+sci), (ii) site of *M. intertexta* (si+sci), (iii) site of *M. muricoleptis* (sm) and (iv) site of *M. granadensis* (sg+sig); the second analysis (5 levels) differed from the first by the distinction of allopatric sites (12) from sympatric sites

for *M. ciliaris* and *M. intertexta*. For only those where the null hypothesis is rejected a Fisher's LSD test was calculated. The hypothesis is null when the F test (MSA/MSE) of ANOVA is greater than 1 (p < 0.05).

For the qualitative variables, environmental profiles were established for each taxon having more than five sites; the classes of each variable are represented in Table 4. *M. ciliaris*: Algeria: 5.776 - 10.464 - 10.465 - 10.642 - 10.643 - 10.644 - 10.645 - 10.648 - 10.792 - 10.932 - 10.933 - 10.934 - 10.935 - 10.936 - 11.571 - 11.574 - 11.575 - 11.576 - 12.547 - 12.551. Crete: 2.081. Cyprus: 23.627 - 23.629 - 23.635 - 23.636. Greece: 2.239. Israel: 2.004 - 7.687 - 7.688. Italy: 2.075 - 5.782 - 22.419 - 22.420. Morocco: 25.506 - 25.506 - 29.024. Portugal: 7.826 - 24.554 - Sardinia 26.016. Sicily: 2.067 - 2.186 - 24.831 - 24.844 - 24.847 - 24.849 - 24.852 - 24.853. Spain: 7.721 - 28.255 - 28.256. Syria: 22.206 - 24.022 - 24.023. Tunisia: 5.785 - 5.786 - 7.714 - 7.723 - 10.637 - 18.460 - 18.463 - 18.464 - 20.104 - 21.802 - 21.803 - 21.804. Turkey: 23.932.

M. intertexta: Algeria: 4.010 - 10.647 - 10.649 - 10.650 - 10.651 - 10.652 - 10.659 - 10.788 - 10.791 - 11.579 - 11.580 - 11.909 - 11.910 - 11.911 - 11.912 - 15.748. Cyprus: 2.367 - 2.370 - 5.777. Egypt: 13.797 - 13.799. France: 2.044 - 2.045. Israel: 1.589 - 2.363 - 2.364 - 2.366 - 5.779. Italy: 2.071 - 2.375. Jordan: 18.084 - 19.002. Morocco: 10.662. Portugal: 2.372 - 2.377 - 5.783 - 8.323. Sardinia: 12.302 - 26.021. Sicily: 24.816 - 24.819 - 24.821 - 24.823 - 24.824. Spain: 1.590 - 2.361 - 2.362 - 28.257. Syria: 13.795 - 13.796. Tunisia: 4.008 - 4.011 - 5.489 - 7.724 - 18.465 18.466.

M. granadensis: Israel: 2.359 - 5.778 - 5.822. Syria: 13.818 - 13.819 - 22.207. Turkey: 23.928 - 23.931 - 26.380.

M. muricoleptis: Greece: 22.423. Italy: 15.343 - 22.422. Sicily: 24.825 - 25.026 - 25.027 - 25.002.

Table 2. Collection of SARDI.

		Allopati	ric sites				
Country	M. ciliaris (sc)	<i>M. intertexta</i> (si)	M. muricoleptis (sm)	M. granadensis (sg)	M. ciliaris- M. intertexta (sci)	M. intertexta- M. granadensis (sig)	Total
Algeria	15	11	-	-	5	-	31
Crete	1	-	-	-	-	-	1
Cyprus	4	3	-	-	-	-	7
Egypt	-	2	-	-	-	-	2
Spain	2	3	-	-	1	-	6
France	-	2	-	-	-	-	2
Greece	1	-	1	-	-	-	2
Israel	3	4	-	2	-	1	10
Italy	4	2	2	-	-	-	8
Jordan	-	2	-	-	-	-	2
Morocco	3	1	-	-	-	-	4
Portugal	1	3	-	-	1	-	5
Sardinia	1	2	-	-	-	-	3
Sicily	5	2	4	-	3	-	14
Syria	3	2	-	3	-	-	8
Tunisia	10	4	-	-	2	-	16
Turkey	1	-	-	3	-	-	4
Total	54	43	7	8	12	1	125

Table 3. Number of allopatric and sympatric sites by country.

Collection of rhizobium strains

In a homogeneous site in Algiers (sub-humid, soil with loamy texture and slightly alkaline pH), 8 populations, two by taxon, were planted. The populations of *M. granadensis* and *M. muricoleptis* originate from the Mediterranean collection (SARDI) and those of M. ciliaris and M. intertexta originate from an Algerian collection (ENSA d'Alger). Populations of M. ciliaris (C58) and M. intertexta (I58) from a sympatric site were taken into account. On the generated seedlings, nodules were collected from which a collection of 34 strains of rhizobia was obtained (Table 5). Bacterial isolates were obtained using the crushed-nodule method (Vincent, 1970) from nodules removed from 60 days old plants. The various strains were stored in glycerol at -80 °C, after having been identified by a symbiotic test on M. polymorpha, in addition to the analysis of 16S rRNA sequences (Rome et al., 1996).

Extraction of DNA, Amplification by REP-PCR and method of analysis

The DNA extraction was conducted following the protocol of Abdelguerfi-Laouar (2005). The rep-PCR and BOX-PCR was completed according to the protocol of Rademaker et al. (1998). A primer for BOX-PCR (BOX A1R: 5'-CTACGGCA-AGGCGACGCTGACG-3') and two primers for REP-PCR (REP 1R: 5'-IIIICGICGICATCIGGC-3' and REP 2I: 5'-ICGICTTATCIGGCCTAC-3') were used (Versalovic et al., 1994). The visualization of bands was made by UV on long agarose gels at 1.5 % (0.5X TBE) at 90 V voltage.

The binary data were established on the basis of the presence (1) absence (0) of bands of REP and BOX markers. From these data, a tree of genetic distance was obtained by groupings of plant from a comprehensive comparison of characters, then by calculating the mean distances of grouped plants (NJ method: Neighbour Joining). Support for clustering was determined by bootstrap procedure applied on binary REP-BOX data (1000 replications). The software used is Dissimilarity Analysis and Representation for Windows (DARwin) (Perrier et al., 2003; Perrier & Jaquemoud-Collet, 2006).

Symbiotic Test

The two species S. meliloti and S. medicae represented by a 2011 strain and two ABS7 strains (Bekki et al., 1987) and USDA1827, respectively, were inoculated on the populations of the four taxa of the Intertextae subsection. For symbiotic tests we kept the same host populations that served to obtain the collection of rhizobium. The seeds were disinfected with sulphuric acid for 30 minutes and then germinated after flushing. After germination, each seed was transferred, in sterile conditions, in tubes containing the FAHRAEUS agar medium (1.5 %) and placed in a culture chamber (20 °C, 60 % relative humidity and a photoperiod of 16 h light and 8 h darkness). The inoculation was carried out after 48 h of development of the roots (0.3 ml/seedling). The notations begin after a week and end after 90 days. The experimental protocol used for the symbiotic tests is total randomization with three plants per population.

RESULTS

Frequencies and geographical distribution of taxa

The most frequent taxon of the *Intertextae* subsection in the Mediterranean region is *M. ciliaris* with a frequency of 47.8 % followed by *M. intertexta* (40.6 %) and, finally, *M. granadensis* (6.5 %) and *M. muricoleptis* (5.1 %). Of 17 Mediterranean countries and islands, Algeria has the highest frequency for *M. ciliaris* and *M. intertexta*. From figure 1, which represents the distribution of 121 sites (longitude and latitude data available), *M. muricoleptis* and *M. granadensis* are confined in two restricted and different regions: the North and the East of the Mediterranean, respectively. These two taxa are included in the area of distribution of *M. ciliaris* and *M. intertexta*.

By comparing the presence of taxa by country between the bibliographic data (Table 1) and the collection studied (Table 2, Fig. 1) similarities and differences can be noted. In the Collection SARDI, *M. ciliaris* was not collected in Egypt, Iraq, France, and Lebanon; the same is true for *M. intertexta* in Crete, Greece and Turkey. *M. muricoleptis* was not collected in France and *M. granadensis* was not collected in Egypt, Jordan, and Lebanon. Contrarily to the literature, *M. ciliaris* and *M. intertexta* were collected in Israel; *M. intertexta* was collected in Egypt, Cyprus, and Jordan; these two taxa therefore exist in these countries.

Inter-taxa ecological variability

Rainfall, altitude and pH

The comparison among *M. ciliaris*, *M. intertexta*, *M. muricoleptis* and *M. granadensis* shows that, with regard to altitude and pH, few differences exist (Table 6). Only rainfall allows differentiating between the sites of *M. ciliaris*, *M. intertexta*, sympatric sites (*M. ciliaris* and *M. intertexta*) (Table 7), *M. granadensis* and the sites of *M. muricoleptis*. This latter taxon prefers the highest rainfalls that exceed 800 mm. *M. granadensis* has the lowest rainfall average of all the subsection.

To have an idea about the limits of adaptation of each taxon, minimum and maximum values of each character and each taxon were taken into considerations. *M. intertexta* shows very broad adaptation for its precipitation needs; it shows the biggest amplitude with 850 mm (Fig 2). It manages to grow in dry conditions (150 mm, a single site in Jordan) as well as in very wet conditions (1000 mm), but given the average for this character, it is more frequent in the rainy areas. *M. granadensis* and *M. ciliaris* are the least demanding in terms of precipitation. *M. muricoleptis* is water demanding as it is only found in sites with more than 700 mm of rainfall (Fig 2). In altitude, minimum and maximum values are very close in the four taxa; they vary from 5 to 10 m for low altitudes and from 900 to 1000 m for higher values. Sympatric sites of *M. ciliaris* and *M. intertexta* show an average rainfall closer to the average for *M. intertexta* than to the average for *M. ciliaris*.

Edaphic characters (Texture, drainage, depth of soil and soil reaction)

The soil textures in the sites of the four taxa are fine; they are clayey or clay-loam. The loamy texture is specific only to *M. ciliaris*, *M. intertexta* and *M. granadensis* (Fig. 3). The *Intertextae* taxa are found both in soils with good water drainage and soils where water stagnates on the surface. Only *M. intertexta* shows 100%, in relative frequency, for the class of submerged soil (waterlogged) (Fig. 4) it is the only taxon that was not mentioned in the



Figure 1. Geographical distribution of *Intertextae* subsection taxa (121 sites) by longitude and latitude. The rectangle is 1 x 2 degrees [1 line: North Latitude (N: 31 to 42); 2 column: East Longitude (E: 0 to 38) and West (W: 0 to 10)]. Abbreviations : c, *M. ciliaris*; i, *M. intertexta*; g, *M. granadensis*; m, *M. muricoleptis*.



Figure 2. Minimum, average, and maximum values of annual average rainfalls by taxon.

class with more than 40 cm of depth (Fig. 5). *M. ciliaris* and *M. intertexta* can develop in soils that are not very deep (10 cm). *M. muricoleptis* and *M. granadensis* prefer deep soils.

M. granadensis does not seem to withstand soil salinity considering its total absence in this type of soil (Fig. 6). Owing to the absence of data on soil reaction, M. muricoleptis was not studied. Although this data is qualitative, ecological profiles confirm the resistance of *M. ciliaris* and *M.* intertexta to salts as they are present in various sites qualified as salty. Although the variance analysis did not highlight significant differences for the pH, from ecological profiles of the soil reaction character (Fig. 6) there are preferences depending on the taxon. All of the three taxa M. ciliaris, M. intertexta and M. granadensis grow on alkaline soils. M. granadensis is the only one present in sites with neutral soils and M. intertexta confirms its presence on acid soils. Acid soils, with a pH of 5, characterize only a few sites of M. ciliaris and M. intertexta. By contrast, neutral and alkaline soils are suitable for the four taxa of the subsection.

Slope and Land Use

M. ciliaris and *M. intertexta* prefer flat grounds in contrast to *M. granadensis*, which prefers sloping grounds (16-30%). *M. muricoleptis* is intermediary with a preference for flat to undulating ground (Fig. 7). The four taxa of the *Intertextae* subsection are found in cultivated and grazed soils and only *M. ciliaris*, *M. intertexta* and *M. granadensis* are found on roadsides (Fig. 8). *M. ciliaris* and *M. granadensis* are more frequent on roadsides and *M. intertexta* is more frequent in cultivated lands. Unlike other taxa, *M. muricoleptis* is found more often in pastures.

Symbiotic responses

The endemic taxa, *M. muricoleptis* and *M. granadensis*, just as the taxa of *M. intertexta* and *M. ciliaris*, are exclusively nodulated, in Algeria. The inoculation of 34 trapped strains of rhizobia on *M. polymorpha* showed an efficient nodulation Nod+/Fix+ on all tested plants. This result confirms that all collection of rhizobium is *S. medicae*.



Figures 3–8. Ecological profiles indicate the relative frequency of the taxon in the different classes (for class limits see Table 4). Figure 3: soil texture. Figure 4: soil drainage. Figure 5: depth. Figure 6: soil reaction. Figure 7: slope. Figure 8: land use.

The inoculation of the reference strain 2011 *S. meliloti* shows that on *M. truncatula* (A17) all the plants nodulate and are efficient. No taxon of the *Intertextae* nodulated efficiently with this strain. *M. ciliaris* and *M. intertexta* do not show any nodule. *M. ciliaris* offers the highest percentage of bumps and *M. granadensis* shows the highest percentage of Nod-. In *M. granadensis* and *M. muricoleptis* non efficient nodules were formed on a small number of plants (Fig. 9). The strain ABS7, obtained from *M. ciliaris*, nodulates efficiently, without exception, all the plants of the *Intertextae* subsection.

Variables	Abbreviation	Class codes	Classes
Texture	Tex	Tex1-Tex3	Clay, loamy, loamy-clay
Drainage	Drn	Drn1-Drn4	Good soil drainage, free flow, Stagnation in surface, Submersion
Depth (cm)	Dpth	Dpth1-Dpth3	0-10, 20-40 and +40 cm
Soil Reaction	Rct	Rct1-Rct4	Acidic, Neutral, Alkaline, Saline
Slope (%)	Slp	Slp1-Slp3	0-3 % Flat, 3-8 % hilly, 16-30 % sloping
Land Use	LU	LU1-LU3	Crops, grazing, roadside

Table 4. Upper Limits of qualitative variable classes used for ecological profiles.

Taxon	Population	Code	Altitude (m)	Rainfall (mm)	Origin	Rhizobia Strains
M. ciliaris	DZ.C242	242	980	450	Algeria	3 Strains: DZ.c242.G3.1 ; DZ.c242.G3.2 ; DZ.c242.G3.3
M. ciliaris	DZ.C58	58	860	649	Algeria	3 Strains: DZ.c58.G1.2, DZ.c58.G1.4, DZ.c58.G1.5
M. intertexta	DZ.I331	331	880	1053	Algeria	5 Strains: DZ.i331.G2.1; DZ.i331.G2.2; DZ.i331.G2.3 ; DZ.i331.G2.4 ; DZ.i331.G2.5
M. intertexta	DZ.I58	58	860	649	Algeria	5 Strains: DZ.i58.G2.1, DZ.i58.G2.2, DZ.i58.G2.3, DZ.i58.G2.4 DZ.i58.G2.5
M. granadensis	DZ.GAUS98	5.822	200	550	Israel	5 Strains: DZ.GAUS98.1.1, DZ.GAUS98.1.2, DZ.GAUS98.1.3, DZ.GAUS98.1.4, DZ.GAUS98.1.5,
M. granadensis	DZ.GAUS105	23.928	950	600	Turkey	5 Strains: DZ.GAUS105.1.1, DZ.GAUS105.1.2, DZ.GAUS105.1.3, DZ.GAUS105.1.4, DZ.GAUS105.1.5
M. muricoleptis	DZ.MAUS.110	24.825	630	800	Sicily	5 Strains: DZ.MAUS.110.1.1, DZ.MAUS.110.1.2, DZ.MAUS.110.1.3, DZ.MAUS.110.1.4, DZ.MAUS.110.1.5
M. muricoleptis	DZ.MAUS.107	25.028	5	700	Italy	3 Strains: DZ.MAUS.107.1.1 DZ.MAUS.107.1.3, DZ.MAUS.107.1.4

Table 5. Collection of 34 rhizobia of Intertextae obtained by trapping.

Factors	Rainfall		Altit	tude	pН	
Sites	Number of sites	Average (mm)	Number of sites	Average (m)	Number of sites	Average
M. ciliaris	34	585.88 b	34	278.24	16	7.98
(sc+sc1)					-	
M. intertexta	30	621.83 b	30	249.50	8	7.73
(si+sci)						
M. granadensis	5	530.00 b	5	398.00	4	7.90
(sg)						
M. muricoleptis	7	835.71 a	7	333.57	3	7.17
(sm)						
Meaning	-	* *	-	ns	-	ns
LSD = 1.996						

Table 6. Comparison between environmental factors of the sites of *Medicago ciliaris*, *M. intertexta*, *M. granadensis* and *M. muricoleptis*. The letters a and b: indicate average groups. P(t), t-test on the groups of average [***: P < 0.001; *: P < 0.001; *: P < 0.05; n.s: P > 0.05].

Factors	Rainfall		Alti	tude	pH	
Sites	Number of sites	Average (mm)	Number of sites	Average (m)	Number. of sites	Average
M. ciliaris (sc)	22	571.82 b	22	288.41	13	7.84
<i>M. intertexta</i> (si)	18	628.61 b	18	242.78	5	7.20
M. granadensis (sg)	5	530.00 b	5	398.00	4	7.90
<i>M. muricoleptis</i> (sm)	7	835.71 a	7	333.57	3	7.17
Mixed M. ci-M. int (sm)	12	611.67 b	12	259.58	3	8.60
Meaning	-	* *	-	ns	-	ns
LSD = 1.98						

Table 7. Comparison between environmental factors (3) of sites with *Medicago ciliaris* alone, *M. intertexta* alone, *M. granadensis*, *M. muricoleptis* and mixed sites with *M. ciliaris* and *M. intertexta*. The letters a and b: indicate average groups. P(t), t-test on the groups of average [***: P < 0.001; *: P < 0.01; *: P < 0.05; n.s: P > 0.05].

By contrast, USDA1827 (obtained from *M. truncatula*) nodulates neither *M. muricoleptis* nor *M. granadensis*. It nodulates one population of each taxon of *M. intertexta* and *M. ciliaris*. Indeed, the USDA1827 is specific within the species *M. truncatula*, for it nodulates some populations and not others.

Genetic diversity of the trapped rhizobia

The electrophoresis of rep-PCR and BOX-PCR revealed 114 bands (we only took intense bands) of which 45 are monomorphic and the remainder is made up of polymorphic bands. Polymorphism is more frequent in *M. granadensis* strains with 55.5%

of polymorphic bands, followed by *M. intertexta* strains (50%); it is less than 25% in the other taxa. We did not find specific bands that differentiate the strains of the 4 taxa. Genetic distances (Fig. 10) show a structuring of the strains of *S. medicae* by forming 2 different groups. The first group (G1) corresponds to the strains that nodulate *M. ciliaris* and *M. intertexta* and the second group (G2) is the one trapped in *M. granadensis* and *M. muricoleptis*. There are 2 strains of *M. granadensis* of the population DZ.GAUS98, which come together with those of *M. intertexta*. Symbiotic specificity is clearly established among widespread taxa and endemic ones.

DISCUSSION

Geographic distribution

In the Mediterranean Basin, the four taxa, which have a close relationship (Rose et al. 1988), show different types of distributions: (i) endemic and rare represented by *M. granadensis* and *M. muricoleptis*, (ii) widespread and common represented by *M. ciliaris* and *M. intertexta*. These results confirm those obtained by several authors (Table 1). The new data is that *M. ciliaris* and *M. intertexta* are present in other countries that were never mentioned before in the literature. *M. ciliaris* is present in Israel and *M. intertexta* in Israel, Egypt, Cyprus, and Jordan. *M. ciliaris* is the most frequent in the Mediterranean basin and particularly in Algeria. In this country, this taxon is ranked fifth (163 sites/564) (Abdelguerfi, 2002).

Contrary to *M. ciliaris*, in the south west of the Mediterranean *M. intertexta* is rare; it is absent in the Northwest of Algeria and in the arid areas (Abdelguerfi-Laouar et al., 2003). *M. muricoleptis* is present in very restricted areas, Toulon in France and Sicily in Italy. Nevertheless (according to Coulot & Rabaute, 2013), the presence of *M. muricoleptis* in Toulon (France) is accidental, endemic in the north Mediterranean, from the south of Italy to Greece and *M. granadensis* is more confined to western Asia, endemic in the East of the Mediterranean and only adventitious in Europe and Chile. Contrary to what mentioned by Small et al. (1999), *M. granadensis* coexists with *M. intertexta* as the two taxa were found together on a site.

Ecological requirements

M. muricoleptis is the most demanding water of the *Intertextae*. Its geographic distribution corresponds perfectly with this need. In Morocco, *M. intertexta* and *M. ciliaris* were found only in the areas with high rainfall and are confined to the areas with mild winter and summer (Bounejmate et al., 1992a, 1992b; Bounejmate, 1996). This does not reflect their requirement in the Mediterranean basin.

The Intertextae is known for its preference for heavy and humid soils (Small et al., 1999). In Algeria, M. ciliaris, M. intertexta, M. rigidula and M. scutellata prefer heavy soils and M. ciliaris grows particularly on marly soils (Abdelguerfi, 2002). Considering the specificity of the texture of the Intertextae, the taxa should withstand soils with poor water drainage. Indeed, the results show that the taxa are found both in soils with good water drainage and soils where water stagnates on the surface. M. intertexta is the most adapted to hydromorphic soils, is considered one of the most tolerant to waterlogging of the Annual Medicago genus (Francis & Poole, 1973). The waterlogging and salinity are associated characters in the Mediterranean basin (Kepner et al., 2005). The tolerance of plants to this condition is due to a combination of anatomical, physiological and morphological adaptations (Predeepa-Javahar, 2012). Only M. ciliaris and M. *intertexta* are present in salt soil. Therefore we may conclude that these two taxa are adapted to salinity. Indeed, genotypes originating from salty soils are often considered as tolerant, that is the conclusion of Ben Salah et al. (2010) when they compared under saline conditions (100 mmol of NaCl) two pure lines originating from two different sites, one rich in salt, and the other poor.

In Morocco, Bounejmate et al. (1992a; 1992b; 1994) and Bounejmate (1996) found the two taxa in soils low in phosphorous, hydromorphic and salty. According to Abdelkrim (1995), *M. ciliaris* is found in fallows and idle lands with soils that are colluvial, heavy, mostly marly and clayey, poorly drained and more or less salty. *M. ciliaris* is classified as glycophyte and found in association with halophyte species (Abdelly et al., 2006; Merabet et al., 2006; Barret-Lennard & Setter, 2010). Other research confirmed the adaptation of these taxa to salinity (Greenway & Andrew, 1962; Lachaal et al., 1995; Chérifi et al., 2011).

MERIEM LAOUAR & AÏSSA ABDELGUERFI



Figure 9. Symbiotic responses to the inoculation of the reference strain 2011 *S. meliloti* in the taxa of the Intertextae subsection. Abbreviations : Nod +, nodulating phenotype; Nod + /fix+, nodulating phenotype-fixer; Nod-, non nodulating phenotype; Bump, non nodulating root bulge.



Figure 10. Un-weighted neighbour-joining tree based on the simple matching dissimilarity matrix of REP and BOX markers across the 34 strains of rhizobia trapped in a common site to all taxa in the subsection *Intertextae*. The numbers on the tips indicate bootstrap values (expressed in percentages) and are shown for all clusters with >50 % bootstrap support. Abbreviations: c, *M. ciliaris*; i, *M. intertexta*; m, *M. muricoleptis*; g, *M. granadensis*.

Furthermore, some studies have shown that the frequency of *M. intertexta* diminishes when the pressure of pasture increases (Piano, 1987). Piano et al. (1991) noted that *M. ciliaris* and *M. intertexta* are more frequent in cultivated lands.

Symbiotic response of *M. granadensis* and *M. muricoleptis* in an exotic site

The various populations of *M. granadensis* and *M. muricoleptis* nodulated efficiently in the Algerian common site. The fact that *M. granadensis* and *M. muricoleptis* nodulated efficiently with rhizobia natives of Algeria, shows that their absence is not related to a restrictive coevolution plant-rhizobium in this region. On some species, particularly cultivated ones, a clear evolution was noted between the species and its symbiote and one of the more interesting examples is the pea, which may form an efficient symbiosis with strains of rhizobium from its site of origin in Turkey, but not with strains from other parts of this country (Lie et al., 1987).

Taxonomic determination of the trapped collection of *Rhizobia*

M. muricoleptis, M. granadensis, M. intertexta and *M. ciliaris* are exclusively nodulated by the strains of S. medicae, which confirms the results of Béna et al. (1998) on the Intertextae subsection and the results of Zribi et al. (2007) on M. ciliaris. Indeed, in the trapping site, there were different species of *Medicago* that were only nodulated by S. meliloti including species of the Melilotus Mill. genus. This confirms the specificity between the subsection of the Intertextae and S. medicae. A recent study (Epstein et al., 2012), comparing the two genomes of S. medicae and S. meliloti, showed that horizontal exchanges were almost exclusively of plasmid genes and that the divergence between the two species resulted from episodes of recent selection pressures.

Maybe it is not very correct to believe that this specificity, whether for *M. polymorpha* or the subsection of the *Intertextae*, is related only to the host. Indeed, other mechanisms affect the chemical structure of the synthesized Nod factors and therefore prevent the nodulation (Van Rhijin & Vanderleyden, 1995; Dénarié et al., 1996; Long, 1996). Alfalfa, for example, is only nodulated in the presence of sulphated Nod factors. This specificity mechanism is based on the presence or absence, depending on bacterial strains, of some genes that control the synthesis of specific substitutes or their transfer on the common skeleton. Other than genetic factors, exogenous factors can influence the symbiosis either for the choice of rhizobia that nodulate the plant or for their efficiency.

According to Garan et al. (2005), *S. medicae* nodulate more the species of *Medicago* that are adapted to acid soils; by contrast, *M. meliloti* forms a more frequent symbiosis with those that grow on alkaline to neutral soils. This result does not apply to the 4 taxa of the *Intertextae* subsection, since they rather prefer alkaline to neutral soils.

Symbiotic specificity

The symbiotic response is not limited to the presence or absence of nodules. Others structures can exist and appear visually on the roots in the form of more or less developed protuberances. Generally, these forms do not grow bigger in length, in fact, their development stops quickly. The study of the effect of the Nod factors of *Rhizobium meliloti* (equivalent to *S. meliloti*) on cultivated alfalfa revealed several structures, such as nodules and other protuberances (Grosjean & Huguet, 1997).

Therefore, the symbiotic response other than the presence absence of efficient nodules cannot be dissociated from genetic variations of the host plant. In this sense, the Nod- of *M. muricoleptis* and *M. granadensis* induced by *S. meliloti* shows the difference of these taxa relative to *M. ciliaris* and *M. intertexta*.

Furthermore, the strain USDA1827 shows a specificity of inter- and intra-species host and it is not the only one. The population ES103 of *M. polymorpha* achieves an efficient symbiosis with ABS7m, while the population F34003 does not form any nodule with this same strain (Béna et al., 1998).

Genetic relation between rhizobium and taxa

According to Martínez -Romero (2009), plantrhizobium coevolution is more oriented by the plant than by the rhizobium; it is the plant that must constantly select the strains with which it gets in symbiosis. This result shows that the taxa acquired genetic differentiation. In Tunisia, the results of molecular and enzymatic analysis did not show strains-species specificity in indigenous strains of *M. sativa* cv. Gabbès, *M. scutelleta* cv. Kelson, *M. truncatula* and *M. ciliaris* (Jebara, 2001). Although *M. muricoleptis* and *M. granadensis* do not share the same areas of distribution, they select genetically close strains of rhizobium that are different from the ones of indigenous taxa, *M. ciliaris* and *M. intertexta*.

These results indicate cospeciation between the rhizobia and the endemic and widespread taxa. Cospeciation between the rhizobia and leguminous plants is not clearly established. Some authors indicate that there is little concordance between the phylogeny of bacteria and the phylogeny of legumes plants (Doyle, 1998; Béna et al., 2005); others, on the contrary, find links between both partners (Ueda et al., 1995; Wernegreen & Riley, 1999; Laguerre et al., 2001).

What is the effect of the two factors on the geographical distribution of taxa?

When analyzing ecological data, it appears that there are no conditions specific to either taxon. Some variations were observed among taxa but are not discriminating. Consequently, M. muricoleptis and M. granadensis are not specific to some habitats and the limit of their dispersion is not ecological. According to Griggs (1940), climatic and edaphic factors are not the primary causes of the rarity of a species, but the primary cause is competitiveness. Other biotic factors may come into play; we have seen that symbiosis does not seem to be a factor that limits the dispersion of endemic species since they have grown and nodulated in Algeria where they are naturally absent. Indeed, from a study on the effect of symbiosis on the expansion of the Medicago genus (Béna et al., 2005), it was concluded that symbiotic specificity can influence the distribution of the species of the Medicago genus, but not to the point of influencing their level of endemism.

In this case, *M. muricoleptis* and *M. granadensis* show local endemism that generally results from geographic and genetic isolation. The geographical distribution of the 4 taxa may result from allopatric

speciation where the populations are first separated geographically, which provokes a net decrease or a stop of the genetic exchanges, and the progressive accumulation of differences among the populations.

According to the phylogenetic results, that indicate that *M. ciliaris* and *M. intertexta* evolved from a common lineage and that *M. intertexta* evolved independently and gave rise to *M. muricoleptis* and *M. granadensis* (Rose et al., 1988), we may assume that *M. muricoleptis* and *M. granadensis* are young species (neoendemism) (Willis, 1922; Stebbins, 1980) compared with *M. intertexta* and *M. ciliaris* and that over time they could extend to other areas. As mentioned by Lamotte (1994), in allopatric speciation, populations may be confronted to different environments allowing or provoking differential adaptations.

CONCLUSION

Ecological factors did not explain why phylogenetically close taxa belonging to the same subsection have different geographical distributions, one narrow and one wide. The ecological requirements of taxa are more or less similar demonstrating that this is not the factor limiting the distribution of M. muricoleptis and M. granadensis in the region of the Mediterranean. Either the symbiosis is not a factor limiting the presence of these taxa which perform an efficient symbiosis in an area where are naturally absent. As against, endemic and widespread taxa show selectivity in symbiont (rhizobia strains) which confirms their differences. Other factors, such as genetic pool of taxa, should be investigated to clarify the distribution of the Intertextae.

ACKNOWLEDGMENTS

We thank S.J. Hughes (SARDI) for sending us the ecological data and the collection of *Intertextae*, T. Huguet (ENSA Toulouse, now retired) for helping us in achieving the symbiotic part, G.G. Guittonneau (Univ. Orleans, now retired) for his guidance and advice. This work was supported in part by the Algerian-French cooperation (Tassili project).

REFERENCES

- Abdelguerfi A., 2002. Ressources Génétiques d'Intérêt Pastoral et/ou fourrager: Distribution et Variabilité chez les Légumineuses Spontanées (*Medicago*, *Trifolium*, *Scorpiurus*, *Hedysarum* et *Onobrychis*) en Algérie. Thèse de Doctorat d'Etat, INA, Alger.
- Abdelguerfi A., Chapot J.Y. & Conesa A.P., 1988. Contribution à l'étude de la répartition des luzernes annuelles spontanées en Algérie selon certains facteurs du milieu. Fourrages, 113 : 89–106.
- Abdelguerfi-Laouar M., 2005. Diversité Eco-Génétique des Fabacées et de leurs Symbiotes: Cas de la Section des *Intertextae* du genre *Medicago* L. Thèse de Doctorat, INA, Alger.
- Abdelguerfi-Laouar M., Abdelguerfi A., Bouznad Z. & Guittonneau G.G., 2003. Autoécologie et distribution du complexe d'espèces *Medicago ciliaris-M. intertexta* en Algérie. Acta Botanica Gallica, 150: 253– 265.
- Abdelkefi A., Boussaid M. & Merrakchi M., 1992. Prospection et inventaire des espèces spontanées du genre *Medicago* L. en Tunisie. In: Complexes d'Espèces, Flux de Gènes et Ressources Génétiques des Plantes. Colloque Int. en Hommage à J. Pernès, 8-10/1992, Paris (France), pp. 628-629.
- Abdelkrim H., 1995. Contribution à l'étude des groupements de mauvaises herbes des cultures du secteur algérois : approches synthonomique et phénologique. Thèse de Doc. en Sciences, Univ. Paris XI (Centre d'Orsay).
- Abdelly C., Barhoumi Z., Ghnaya T., Debez A., Ben Hamed K., Ksouri R., Talbi O., Zribi F., Ouerghi Z. & Smaoui A., 2006. Potential utilization of halophytes for the rehabilitation and valorization of saltaffected areas in Tunisia. Biosaline Agriculture and Salinity Tolerance in Plants, 3: 163–172.
- Aitken Y. & Davidson B.R., 1954. pH and drought in relation to the dominance of subterranean clover or annual medics in Mallee an Wimmera soils; notes on pot tests at Melbourne. Journal of Australian Institute of Agriculture Science, 20: 253–256.
- Andrew W.D. & Heley F.W., 1960. Frequency of annual species of *Medicago* on the major soil groups of the Macquarie region of New South Wales. Australian Journal of Agriculture Research, 2: 705–714.
- Barret-Lennard E, Setter T. 2010. Developing saline agriculture: moving from traits and genes to systems. Functional Plant Biology, 37: 3–4.
- Bekki A., Trinchant J.C. & Rigaud J., 1987. Nitrogen fixation (C2H2 reduction) by *Medicago* nodules and bacteroïds under chloride stress. Physiology Plant, 71: 61–67.
- Béna G., Prosperi J.M., Lejeune B. & Olivieri I., 1998. Evolution of annual species of the genus *Medicago*:

a molecular phylogenetic approach. Journal of Molecular Evolution, 9: 552–559.

- Béna G.L., Yet A., Huguet T. & Olivieri I., 2005 Medicago-Sinorhizobium symbiotic specificity evolution and the geographic expansion of Medicago. Journal of Evolution Biology, 1–12.
- Ben Salah I., Slatni T., Gruber M., Messedi D., Gandour M., Benzarti M., Haouala R., Zribi K., Ben Hamed K., Perez-Alfocea F. & Abdelly C., 2010. Relationship between symbiotic nitrogen fixation, sucrose synthesis and anti-oxidant activities in source leaves of two *Medicago ciliaris* lines cultivated under salt stress. Environmental and Experimental Botany, 70: 166–173.
- Bounejmate M., 1992. Distribution des luzernes annuelles spontanées au Maroc en relation avec certains facteurs climatiques et édaphiques. Al Awamia, 79: 17–34.
- Bounejmate M., 1996. Le point sur les travaux réalisés en amélioration des plantes sur les luzernes annuelles par le programme fourrage de l'Institut National de la Recherche Agronomique du Maroc. Cahiers Options Méditerranéennes, 18: 53–64.
- Bounejmate M., Beale P.E. & Robson A.D., 1992a. Annual Medicago Species in Morocco. I. Species and their abundance. Australian Journal of Agriculture Research, 43: 739–749.
- Bounejmate M., Robson A.D. & Beale P.E., 1992b. Annual *Medicago* species in Morocco. II. Distribution in relation to soil and climate. Australian Journal of Agriculture Research, 43: 751–763.
- Bounejmate M., Birouk A. & Tazi M., 1994. Rôle potentiel des légumineuses fourragères et pastorales spontanées au Maroc. In: Tazi M. & El Gharbaoui A. (Eds.). Amélioration Pastorale et Production Fourragère dans le Maghreb. Séminaire Régional, Meknès (Maroc) 19-20/02/1993, pp. 39–49.
- Chérifi K., Boubaker H., Msanda F., Saad B., Boufous E. & El Moussadik A., 2011. Variability of salt tolerance during germination in *Medicago ciliaris* et *M. polymorpha*. Int. Reas. Journal of Plant Science, 2: 201–208.
- Coulot P. & Rabaute P., 2013. Monographie des Leguminosae de France. Tome 3 Tribu des Trifolieae. Bulletin de la Société Botanique du Centre-Ouest, Nouvelle Série, Numéro spécial 40, 760 pp.
- Dangeart P.A., 1926 Recherches sur les tubercules radicaux des légumineuses. Le botaniste, Ser.16, Paris, 270 pp.
- Davis P.H., 1965–1988. Flora of Turkey and the East Aegean Islands. Edinburgh University Press, Edinburgh, 10 vols..
- Dénarié J., Debellé F. & Promé J.C., 1996. Rhizobium lipochitoolosaccharide nodulation factors : signaling

molcules mediating recognition and morphogenesis. Annual Review of Biochemistry, 65: 503–535.

- Dobignard A. & Chatelain C., 2012. Index synonymique de la flore d'Afrique du Nord Volume 4: Dicotyledoneae: Fabaceae-Nymphaeaceae. Éditions des Conservatoire et Jardin Botaniques, Genève, 431 pp.
- Doyle J.J., 1998. Phylogenetic perspectives on nodulation: evolving views of plants and symbiotic bacteria. Trends in Plant Science, 3: 473–478.
- Ehrman T. & Cocks P.S., 1988. Co-adaptation and geographical distribution of reproductive attributes in syrian pastures. In: Pasture, Forage and Livestock Program. Annul Report 1988 ICARDA. Aleppo (Syria), pp. 66–74.
- Ehrman T. & Cocks P.S., 1990. Ecogeography of annual legumes in Syria: distribution patterns. Journal of Applied Ecology, 27: 578–591.
- Epstein B., Branca A., Mudge J., Bharti A.K. & Briskine R., 2012. Population Genomics of the Facultatively Mutualistic Bacteria *Sinorhizobium meliloti* and *S. medicae*. PLoS Genet 8(8): e1002868. doi:10.1371/ journal.pgen.1002868
- Francis C.M. & Poole M.L., 1973. Effect of waterlogging on the growth of annual *Medicago* species. Australian Journal of Experimental Agriculture and Animal Husbandry, 13: 711–713.
- Garan G., Reeve W.G., Bran L., Deiana P., Yates R.J., James D., Tiwari R., O'Hara G.W. & Hawieson J.G., 2005. The symbiotic requierements of different *Medicago* spp. suggest the evolution of *Sinorhizobium meliloti* and *S. medicae* with hosts differentially adopted to soil pH. Plant Soil, 276: 263–277.
- Gouet J.P. & Pilippeau G., 2002. Comment interpréter les résultats d'une analyse de variance? STAT-ITCF. ITCF, Paris.
- Greenway H. & Andrew W.D., 1962. A screening technique to predict field behaviour of medics on saline soils. Australian Journal of Experimental Agriculture and Animal Husbandry, 2: 234–235.
- Griggs R.F., 1940. The ecology of rare plants. Bulletin of the Torrey Botanical Club, 67: 575–594.
- GRIN (Germplasm Resources Information Network), 2014. National Germplasm Resources Laboratory, Beltsville, Maryland [Online Database]. Available from www.ars-grin.gov/cgi-bin/npgs/html/taxon. pl?23564 [accessed 14 July 2014].
- Grosjean C. & Huguet T., 1997. A persistent meristem is formed in nodular structures elicited by Nod factor or by a *Rhizobium meliloti* exopolysaccharide mutant in alfalfa plants which nodulate spontaneously. Plant Science, 127: 215–225.
- Heyn C.C., 1963. The annual species of *Medicago*. Scripta Hierosolymitana, 12: 154.

- Jahandiez E. & Maire R., 1931–1941. Catalogue des plantes du Maroc. (Maroc) [lists as *M. ciliaris* (L.) Krocker].
- Jauzein Ph., 1995. Flore des champs cultivés. INRA Editions/SOPRA, Paris et Vélizy-Villacoublay, 898 pp.
- Jebara R.M., Aouani M.E., Ghrir R. & Mars M., 2001. Genetic diversity of *Sinorhizobium* populations recovered from different *Medicago* varieties cultivated in Tunisian soils. Canadian Journal of Microbiology, 47: 139–147.
- Kepner W.G., Rubio J.L., Mouat D.A. & Pedrazzini F. (Eds.), 2005. Desertification in the Mediterraeaan Region. A Security Issue (1 pp). NATO Security through Science Series, Volume 3, Springer Publishers, The Netherlands. ISBN HB 1-4020-3758-9, 605 pp. (http://www.epa.gov/nerlesd1/land-sci/desert/ images/flyerkepner.pdf)
- Lachaal M., Abdelly C., Soltani A., Hajji M. & Grignon C., 1995. Réponses physiologiques quelques légumineuses spontanées et cultivées à la contrainte saline. In : Facteurs limitant la fixation symbiotique de l'azote dans le Bassin Mediterranéen. 6-8 Avril 1994, Montpellier (France). Eds. INRA, Paris. Les Colloques, 77: 94–109.
- Laguerre G., Nour S.M., Macheret V., Sanjuan J., Drouin P. & Amarger N., 2001. Classification of rhizobia based on nodC and nifH gene analysis reveals a close phylogenetic relationship among *Phaseolus vulgaris* symbionts. Microbiology, 147: 981–993.
- Lamotte M., 1994. Théorie actuelle de l'évolution. Edit. Hachette, Paris.
- Lesins K.A. & Lesins I., 1979. Genus *Medicago* (Leguminosae). A taxogenetic study. Dr W Junk bv, The Hague, The Netherlands, 132 pp.
- Lie T.A., Göktan D., Engin M., Pijnenborg J. & Anlarsal E., 1987. Co-evolution of the legume-*Rhizobium* association. Plant and Soil, 100: 171–181.
- Long S.R., 1996. *Rhizobium* symbiosis: Nod factors in perspective. Plant Cell, 8: 1885–1889.
- Martínez-Romero E., 2009. Coevolution in *Rhizobium*legume symbiosis? DNA Cell Biology, 28: 361–70.
- Meikle R.D., 1977-1985. Flora of Cyprus. Kew: Royal Botanic Gardens.
- Merabet C., Bekki A., Benrabah N., Bey M., Bouchentouf L., Ameziane H., Rezki M., Domergue O., Cleyet-Marel J. & Avarre J., 2006. Distribution of *Medicago* species and their microsymbionts in a saline region of Algeria. Arid Land Research and Management, 20: 219–231.
- Mouterde P., 1966. Nouvelle flore du Liban et de la Syrie. Dar El– Machreq, Beyrouth.
- Perrier X. & Jacquemoud-Collet J.P., 2006. DARwin software http://darwin.cirad.fr/.
- Perrier X., Flori A. & Bonnot F., 2003. Data analysis methods. In: Hamon P., Seguin M., Perrier X. &

Glaszmann J. C. (Eds.). Genetic diversity of cultivated tropical plants. Enfield, Science Publishers. Montpellier, pp. 43–76.

- Piano E., 1987. Some ecology aspects of the distribution of annual medics and subterranean clovers in Sardinia (Italy). In: Annual Self-Reseeding Legumes for Mediterranean Pastures. ISCF, Lodi, 1987, pp. 3–9.
- Piano E., Pusceddu S. & Spanu F., 1991. Observations on the distribution and ecology of annual medics and subterranean clovers in Sicily (Italy). In: 4th Int. Proceedings of Rangeland Congress, 22-26 Avril 1991, Montpellier (France)1, pp. 405–409.
- Pignatti S., 1982. Flora d'Italia. Edagricole, Bologna.
- Ponert J., 1973. Neue Taxonomische Kombination Kategorien und Taxa vor allem der tiirkischen Arten. Feddes Repert, 83: 617–644.
- Pottier-Alapetite G., 1979-1981. Flore de la Tunisie: Angiospermes-Dicotyledones. Ministère de l'Enseignement Supérieur et de la Recherche Scientifique et le Ministère de l'Agriculture, Tunisie, 1190 pp.
- Prosperi J.M., Gensollen V., Olivieri I. & Mansat P., 1989. Observation sur la répartition et l'écologie de luzernnes annuelles et de trèfle souterrain en Corse. In : 16th International Grassland Congress, 4–11 Oct.1989, Nice (Fr). 1: 295–296.
- Predeepa-Javahar R., 2012. Physiological and Anatomical Implications of Salinity on Rice as a Semi-Aquatic Species, Cambridge Scholars Publishing. Copyright © 2012 ISBN (10): 1-4438-4098-X, ISBN (13): 978-1-4438-4098-9
- Quézel P. & Santa S., 1962. Nouvelle flore de l'Algérie et des régions désertiques méridionales. CNRS, Paris, 1170 pp.
- Rademaker J.L.W., Louws F.J. & De Bruijin F.J., 1998. Characterization of the diversity of ecologically important microbes by rep-PCR genomic fingerprinting. Molecular Microbial Ecology Manual, 3.4.3: 1–27.
- Rome S., 1996. Diversité génétique et fonctionnelle des sinorhizobia isolés de *Medicago truncatula*. Thèse doctorat, Université Claude Bernard.
- Rome S., Fernandez M.P., Brunel B., Normand P. & Cleyet-Marel J.C., 1996. *Sinorhizobium medicae* sp. nov., isolated from annual *Medicago* spp. International Journal of Systematic Bacteriology, 46: 972– 980.
- Rose R.J., Schlarbaum S.E., Small E. & Johnson L.B., 1988. Chloroplast genomic variation and phylogeny in *Medicago* section *Intertextae*. Canadian Journal of Botany, 66: 1352–1358.
- Small E., 1981. A numerical analysis of major groupings in *Medicago* employing traditionally used characters. Canadian Journal of Botany, 59: 1553–1577.

- Small E., 2011. Alfalfa and Relatives: Evolution and Classification of *Medicago*. NRC Research Press, Canada, 727 pp.
- Small E. & Jomphe M., 1989. A synopsis of the genus *Medicago* (Leguminosae). Canadian Journal of Botany, 67: 32–71.
- Small E., Crompton C.W. & Brookes B.S., 1981. The taxonomic value of floral characters in tribe Trigonelleae (Leguminosae), with special reference to *Medicago*. Canadian Journal of Botany, 59: 15– 80.
- Small E., Warwick S.I. & Brookes B., 1999. Allozyme variation in relation to morphology and taxonomie in *Medicago* sect. *Spirocarpos* subsect. *Intertextae* (Fabaceae). Plant Systematics and Evolution, 214: 29–47.
- Smythies B.E., 1984–1986. Flora of Spain and the Balearic Islands: checklist of vascular plants. Englera, 3: 1–882.
- Stebbins G.L., 1980. Rarity of plant species: a synthetic viewpoint. Rhodora, 82 (892): 77–86.
- Täckholm V., 1974. Students' flora of Egypt, ed. 2. Cairo University, 649 pp.
- Townsend C.C. & Guest E., 1966. Flora of Iraq. Volume 3, Leguminales. Ministry of Agriculture, Baghdad, 662 pp.
- Tison J-M., Jauzein Ph. & Michaud H., 2014. Flore de France méditerranéenne continentale. Naturalia publication, 2078 pp.
- Trumble H.C. & Donald C.M., 1938. Soil factors in relation to the distribution of subterranean clover and some alternative legumes. Journal of Australian Institute of Agricultural Science, 4: 206–208.
- Tutin T.G., Heywood V.H. & Burges N.A., 1964–1980. Flora Europea. Cambridge University Press, Vols. 1–5.
- Tutin T.G., Heywood V.H., Burges N.A., Moore D.M., Valentine D.H., Walters S.M., Webb D.A., 1967.Flora Europea. 2: Rosaceae to Umbilliferae. Cambridge Univ. Press, pp. 185–187.
- Ueda T., Suga Y., Yahiro N. & Matsuguchi T., 1995. Phylogeny of sym plasmids of rhizobia by PCR based sequencing of a nodC segment. Journal of Bacteriology, 177: 468–472.
- Van Rhijin P. & Vanderleyden J., 1995. The Rhizobiumplant symbiosis. Microbiology and Molecular Biology Reviews, 59: 124–142.
- Versalovic J., Schneider M., de Bruijin F.J. & Lupski J.R., 1994. Genomic fingerprinting of bacteria using repetitive sequence bases PCR (rep-PCR). Methods in Molecular and Cellular Biology, 5: 25–40.
- Vincent J.M., 1970. A manual for the practical study of the root-nodule bacteria. IBP Handbook N°15. Blackwell Scientific Publications, Oxford, 164 pp.

- Wernegreen J.J. & Riley M.A., 1999. Comparison of the evolutionary dynamics of symbiotic and housekeeping loci: a case for the genetic coherence of rhizobial lineages. Molecular Biology and Evolution, 16: 98– 113.
- Willis J.C., 1922. Age and area: a study in geographical distribution and origin of species. Cambridge, Uni-

versity Press, 8 vol. + 260 pp.

- Zohary M. & Feinbrun-Dothan N., 1966. Flora Palestina. Jerusalem.
- Zribi K., Badri Y., Saidi S., Van Berkum P. & Aouani M.E., 2007. *Medicago ciliaris* growing in Tunisian soils is preferentially nodulated by *Sinorhizobium medicae*. Soil Research, 45: 473–477.