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Natural population of oat crown rust in Tunisia

Imran HAMMAMI[1], Mohamed Bechir ALLAGUI[1], Mohamed CHAKROUN[1] and Mohamed EL-GAZZEH[2]

[1]Laboratoire de Protection des Végétaux and Laboratoire de Production Animale et Fourragère, Institut National de la Recherche Agronomique de Tunisie, 2049 Ariana, Tunisia
[2]Unité de Génétique et Bioressources, Faculté des Sciences de Tunis, Campus Universitaire, 2094 El-Manar, Tunis, Tunisia

Summary. Oat crown rust, caused by *Puccinia coronata* f. sp. *avenae*, is considered the most widespread and damaging disease of oat (*Avena sativa*) in Tunisia. The virulence structure of the natural oat rust population in Tunisia was studied in four areas from 2002 to 2007 using *Puccinia coronata* resistance genes (*Pc-genes*). The areas are located in northern Tunisia: Afareg, Bourbia, Sedjnen and Ariana. In this survey *Pc38*, *Pc39* and *Pc68* showed a high level of resistance to natural oat crown rust. But the most important finding in 2004 in Ariana was that the rust was virulent on *Pc68* (IT '4'). Moreover, in 2002 Sedjnen survey, there was a high degree of virulence to *Pc39* (IT '3'). In the other areas and other years, *Pc68* and *Pc39* were highly resistant to natural oat crown rust. Only *Pc38* showed a stable high level of resistance to natural oat crown rust in all four areas and during the six years of the study. Areas showing a high degree of similarity were Sedjnen and Afereg (SI=4.5). Ariana and Bourbia showed little similarity to the other areas, and had the greatest dissimilarity to each other (SI=11.30). The virulence phenotypes of the *P. coronata* natural population in Tunisia are certainly influenced by the alternate host, *Rhamnus lycioïdes* which is abundant in the mountains of northwestern Tunisia. A combination of the *Pc38*, *Pc39* and *Pc68* genes will provide a high level of durable protection from crown rust in Tunisia.

Key words: *Puccinia coronata* f. sp *avenae*, virulence, *Pc*-genes.

Introduction

Oat crown rust, caused by *Puccinia coronata* f. sp. *avenae*, is the most widespread and damaging disease of oats (*Avena sativa*) in Tunisia (Hammami *et al*., 2006), especially in areas where the relative humidity is high during the growing season. Oats (*Avena sativa* and *A. byzantina*) are the most important livestock feed in Tunisia. It is grown over a wide range of climatic conditions from the humid and sub-humid in the northeast and northwest, to the semi-arid in the southwestern and north-central part of the country. During the last 5 years, on an area of 174,000 ha for oats cultivation, it was estimated that 30% of oats was sown in association with tare, whereas 70% was pure oats, of which 10% was used for seed production (Al Faïz *et al*., 2004). Oat grain has long been known as a high-quality food and feed. It has the highest protein level among the cereals (Peterson, 1992). Furthermore, its amino acids are superior compared with wheat, barly, or maize (Rines *et al*., 2006). A high level of soluble fiber is desired in oats desined for human consumption. Oats in the human diet reduces serum cholesterol levels and slows down increases in blood sugar (Peterson, 2004). Damage to oats caused by fungal diseases (mainly *P. coronata* f. sp. *avenae*) was estimated between 10 to 30% in April 1990 on plants intended for fodder production, and between 30 and 70% on oats intended for grain production (Allalgui and Chakroun, 2000). The most important oat crown rust resistance sources used elsewhere are based on a set of 16 Pc-gene lines exhibiting race-specific resistance (Chong *et*

Corresponding author: I. Hammami
Fax: +216 71 871 666
E-mail: imran.hammami@fst.rnu.tn

al., 2000). However, the resistance of each of these *Pc* race-specific genes has been overcome by rapid shifts in the crown rust virulence pattern after the release of the cultivars containing these genes (Rines *et al*., 2006). In Tunisia, the ever-growing prominence of the alternate host buckthorn, *Rhamnus lycioïdes*, has probably speeded up this shift in virulence (Hammami *et al*., 2006). These 16 single gene oat lines with seedling resistance genes are used to reveal the diversity of the virulence structure of oat crown rust, but there have been few studies to determine the effectiveness of these genes against populations of *P. coronata* indigenous to Tunisia. Oat crown rust surveys have been conducted in Tunisia since 2000. The two principal objectives of these surveys were to furnish data on the year-to-year prevalence of known races and to detect new and potentially dangerous races as soon as possible after their appearance (Simons, 1955). The race surveys also provide information about the *P. coronata* population structure and the epidemiological areas for cereal rust (Leonard *et al*., 1992; Leonard, 2003). The objective of this study was to determine levels of virulence in *P. coronata* populations in Tunisia on 18 crown rust differential lines of oats over a 6-year period from 2002 through 2007.

Materials and methods

Four areas in Tunisia were selected to study the virulence of the natural rust populations and to determine the most resistant *Pc*-gene. The areas are located in northern Tunisia: Afareg (36°40'03.80"N, 9°08'55.31"E); Bourbia (36°36'01.75"N, 10°07'26.15"E); Sedjnen (37°03'27.77"N, 9°14'03.59"E) and Ariana (36°50'37.39"N, 10°11'15.75"E) (Figure 1). A plot with 18 differential oat lines was planted in each area. Each line was planted in a mini-plot consisting of two parallel rows 1.5 m long and 50 cm apart at a rate of 3 g of seed per row. The mini-plots were separated by 2 m in the plot. The lines used had two fundamental qualities: their resistance was mono-genic and all lines had the same reaction to infection by the same oat crown rust isolate. Inspection was started as soon as the first symptoms appeared on the leaves. Several inspections were made over the cropping season. Infection was scored on a scale from 0 to 4 (Chong *et al*., 2000) where 0, no uredinia or other macroscopic signs of infection; ';' no uredinia, but necrotic or chlorotic flecks; 1, small uredinia surrounded by chlorosis or necrosis; 2, small to medium-size uredinia in chlorotic areas; 3, medium-size uredinia in chlorotic areas and

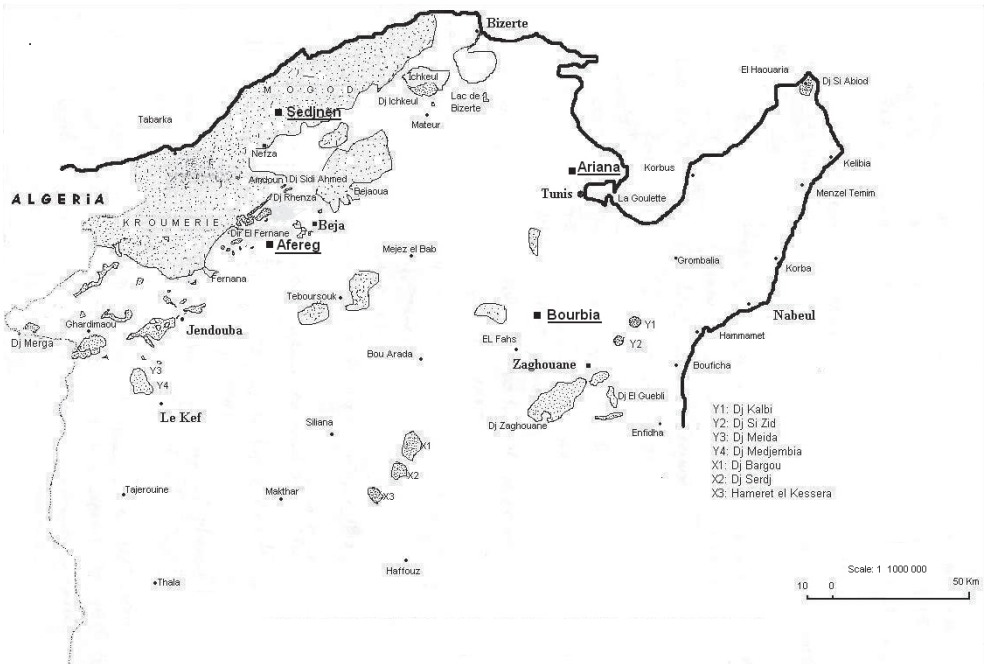

Figure 1. The four regions of Tunisia surveyed and the distribution of buckthorn, represented by dotted surfaces (Shonenberger and Gounot, 1965).

4, large uredinia without necrosis or chlorosis. The percentage of infection was also estimated visually.

The percentages of natural infection of the 18 differential lines from 2002 to 2007 in the four areas were compared in all pair-wise combinations. For these comparisons, a similarity index (SI) was calculated for each pair based on absolute differences in the natural infection percentage on the differential line series:

$$SI = \frac{1}{N}\sum_{i=1}^{N} \mid P_{iA} - P_{iB} \mid$$

where N is the number of host lines in the differential set (=18), P_{iA} the percentage of natural infection in the *i*th differential line in region A, and P_{iB} the percentage of natural infection in the *i*th differential line in region B (Leonard *et al*., 2005, with modification). Thus regions with the same percentage of natural infection in all differential lines would have a SI of 0.0. A greater difference in the percentage of natural infection between a pair of regions indicates a lower similarity of the crown rust population between these regions.

The mean natural infection percentage of the 18 differential lines cultivated in the four areas from 2002 to 2007 was analyzed with the basic module of STATISTICA 6.0 (StatSoft, 2001). The treatment means were compared statistically by Duncan's multiple range tests (Steel and Torrie, 1980).

Results

In all four regions studied, *Pc38* showed a high level of resistance. No pustule emerged during the six years of the study. In some years; 2004, '06, '07 in Ariana, 2003, '06, '07 in Afereg; 2002 in Bourbia and 2002, '03, '04, '06, '07 in Sedjnen, line *Pc38* showed some necrosis in the leaves (Table 1).

Pc68 and *Pc39* also showed a high level of resistance in the four areas during the six years of

Table 1. Reaction[a] of the 18 oat lines to natural crown rust over 6 years, in Ariana, Afereg, Bourbia and Sedjnen.

Oat line	Ariana						Afereg						Bourbia						Sedjnen					
	'02	'03	'04	'05	'06	'07	'02	'03	'04	'05	'06	'07	'02	'03	'04	'05	'06	'07	'02	'03	'04	'05	'06	'07
Pc38	0;	0	;	0	;	;	0	0;	0	0	;	;	;	0	0	0	0	0	;	;	;	0	;	;
Pc39	;12	0	1	;-1	1	1	;	0	;	0	;	0	;	0	;	;1	1	1	;3	;	1	;	1	1
Pc40	3-4	4	4	3	3	4	3	;3	3	4	4	4	4	4	4	4	4	4	3	3-4	4	4	3	4
Pc45	3-4	4	4	4	3	4	1	;	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4
Pc46	3-4	4	4	4	3	4	3	3	4	3	4	4	4	4	4	4	4	4	3-4	4	4	4	4	4
Pc48	3-4	4	4	4	3	3	2	0	2	3	4	4	;2	0	;1	;2	;2	;2	3-4	3	3	3	4	3
Pc50	;3	4	4	2	2-3	3	1	1	2	3	3	3	3-4	4	4	4	4	4	2-3	4	4	4	4	4
Pc51	;2	;1	4	;-1	1	1	3	;	1	2	3	3	2-3	4	4	4	4	4	;2	3	3	3	3	4
Pc52	;2	4	4	1-2	2	2	1	1	2	4	4	4	1-2	4	4	4	4	4	;1	0	2	2	2	2
Pc54	4	4	4	4	3	3	3	3	3	4	4	4	4	4	4	4	4	4	3	4	4	4	4	4
Pc56	;2	4	4	1-2	3	4	1	;	2	3	3	3	1-2	4	4	4	4	4	3-4	4	4	4	4	4
Pc58	2	;1	;2	1	1	2	;	2	1	1	1	;	;2	0	;1	;1	1	1	3-4	;	;	1	1	;
Pc59	;2	3	4	1	0	2	;	2	2	1	3	4	;	0	2	3	3	3	;3	;	2	2	3	3
Pc62	;2	4	2-3	1	0	2	3	2	1	2	2	2	2	0	;2	;2	3	3	;2	4	4	4	4	4
Pc64	2	4	4	3	2-3	2-3	;2	;2	;2	2	4	4	4	3	3	3	3	3	3	4	4	4	4	4
Pc68	0;	0	4	;-1	0	0	0	0;	0	;	0	;	;	0	0	0	;1	;	;	0	0	0	0	;
Pc94	4	4	4	4	3	4	1	1	2	2	3	3	;3	4	4	4	4	4	;	3	3	3	3	4
Pc96	4	4	3	4	3	4	;	;	1	2	3	3	;3	4	4	4	4	4	;4	3	3	3	3	4

[a] 0, no uredinia or other macroscopic signs of infection; ; no uredinia, but necrotic or chlorotic flecks; 1, small uredinia surrounded by chlorosis or necrosis; 2, small to medium-size uredinia in chlorotic areas; 3, medium-size uredinia in chlorotic areas and 4, large uredinia without necrosis or chlorosis.

Table 2. Mean natural crown rust percentage on 18 differential oat lines cultivated in four areas of Tunisia from 2002 to 2007.

Oat line	Ariana	Afereg	Bourbia	Sedjnen
Pc38	0.00 a	0.00 a	0.00 a	0.00 a
Pc39	1.02 a	0.00 a	2.67 ab	3.50 b
Pc40	37.50 a	26.67 b	23.50 c	28.83 d
Pc45	48.33 a	21.67 b	20.00 b	28.83 c
Pc46	35.83 a	35.00 a	31.17 b	32.50 b
Pc48	30.00 a	15.00 b	30.83 a	26.83 c
Pc50	21.83 a	20.00 a	41.83 b	22.67 a
Pc51	13.50 a	28.33 b	38.50 c	25.33 b
Pc52	12.35 a	17.50 b	28.50 c	11.00 a
Pc54	46.67 a	41.67 b	35.17 c	30.00 d
Pc56	26.00 a	19.17 b	37.67 c	22.50 d
Pc58	7.00 a	5.00 a	7.67 a	4.33 a
Pc59	6.02 a	20.00 b	30.00 b	10.00 d
Pc62	7.50 a	17.50 b	26.67 c	14.33 d
Pc64	37.67 a	21.67 b	36.83 a	16.00 c
Pc68	0.03 a	0.00 a	0.83 a	0.00 a
Pc94	6.00 a	20.83 b	20.33 b	15.17 c
Pc96	7.50 a	23.33 b	17.33 c	21.67 bc

[a] Values in line followed by the same letter are not significantly different at 5% probability level.

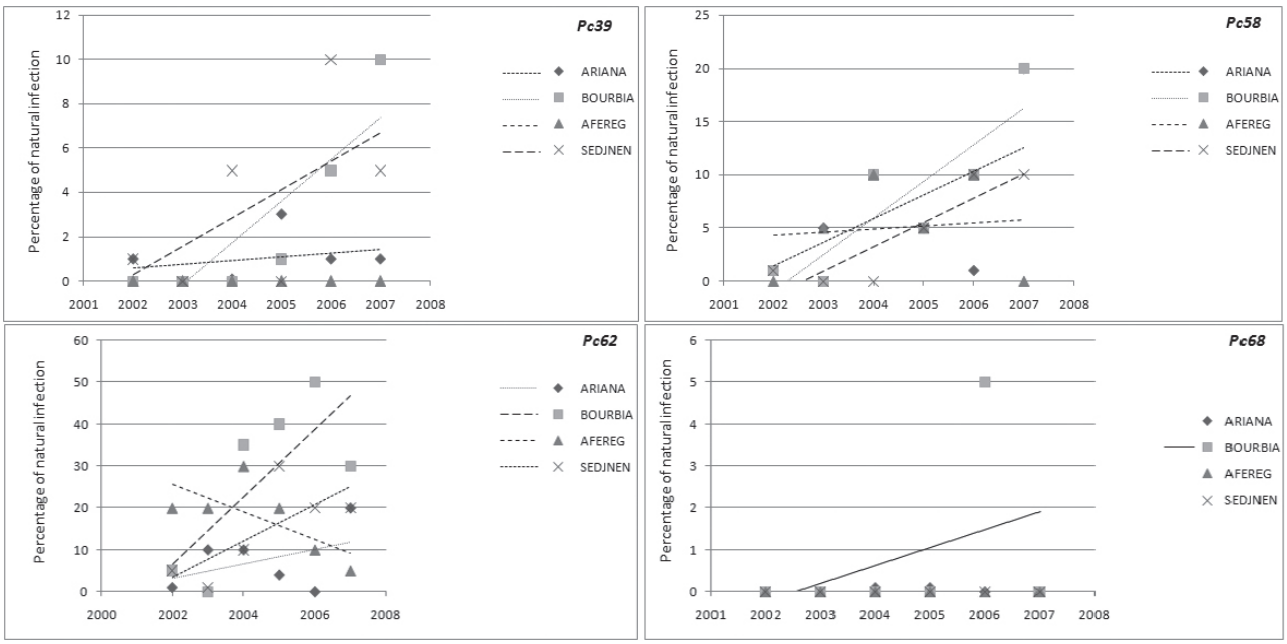

Figure 2. Regression of natural crown rust on the differential oat lines *Pc39*, *Pc58*, *Pc62* and *Pc68* during 2002 to 2007 in Ariana, Afereg, Bourbia and Sedjnen.

Table 3. Average similarity (%) of natural infection on differential oat lines cultivated in Ariana, Afereg, Bourbia and Sedjnen from 2002 to 2007.

Region	Ariana	Bourbia	Sedjnen
Ariana			
Bourbia	11.30		
Sedjnen	7.21	8.74	
Afereg	8.93	7.75	4.51

the study. Although some large uredinia appeared on *Pc68* leaves (IT '4') (Table 1), they represented only a very low percentage of infection (Table 2). In Sedjnen in 2003, medium-size uredinia appeared on *Pc39* leaves (IT '3') (Table 1) but with a low percentage of infection (Table 2). In the other years and areas, *Pc68* and *Pc39* were immune (IT '0') or had a hypersensitive reaction (IT ';') or small uredinia (IT '1') on their leaves. *Pc58* showed a moderate resistance in all areas in most years, but in Sedjnen in 2002, it showed some large uredinia on the leaves (IT '3-4') (Table 1, 2). *Pc62* showed moderate resistance in Ariana, Afereg and Bourbia in all six years, with infection scores varying between ';' and '2', and a maximum mean percentage of infection of 26.67% in Bourbia (Table 2). In Sedjnen, *Pc62* was susceptible to natural crown rust (IT '4') from 2003 to 2007, but in 2002 this line was moderately resistant (IT '2') (Table 1). The remaining lines were generally susceptible to natural crown rust in all areas and during all years, with large uredinia (Table 1) and high rates of infection (Table 2).

From 2002 to 2007, there were significant (P<0.05) increases in the natural crown rust of *Pc39*, *Pc58*, *Pc62* and *Pc68* in Ariana, Afereg and Sedjnen (Figure 2); but not in *Pc62* in Afereg, which showed a lower of percentage of natural crown rust from 2002 to 2007. *Pc68* showed no or only a very low percentage of natural crown rust in Ariana, Afereg and Sedjnen during the six years (Figure 2).

The SI was calculated as the mean absolute difference in the percentage of natural crown rust on the differential line series. Areas with a high similarity were Sedjnen and Afereg (SI=4.51; Table 3). Ariana and Bourbia showed little similarity to any of the other areas (Table 3). The greatest dissimilarity existed between Ariana and Bourbia (SI=11.30; Table 3).

Discussion

All but two (*Pc94* and *Pc96*) of the 18 crown-rust resistance genes represented in the set of differential lines were derived from accessions of the wild oat *A. sterilis* (Hana and Alena, 2008) collected in the Middle East, North Africa and Portugal (Leonard, 2003). Little is known about the crown rust resistance genotypes of commonly grown oat cultivars in Tunisia. In contrast, *Pc38*, *Pc39*, *Pc48*, *Pc58*, *Pc59*, *Pc62*, and *Pc68* have been used in oat cultivars and breeding lines in Canada and the USA (Leonard, 2003). On account of their high resistance to oat crown rust, *Pc38* and *Pc39* were used extensively in Canada and the USA during the 1980s and early 1990s (Chong and Kolmer, 1993; Leonard, 2003). In this survey, *Pc38*, *Pc39* and *Pc68* showed high resistance to natural oat crown rust. But the most important finding was in 2004 in Ariana; here crown rust was virulent on *Pc68* (IT '4') (Table 1) even though the infection percentage was low (data not shown). Moreover, in 2002 in Sedjnen, a noteworthy finding was that crown rust had become virulent on *Pc39* (IT '3') (Table 1). In the other areas and years, *Pc68* and *Pc39* were highly resistant to natural oat crown rust (Table 1 and 2). Genotypes with the *Pc39* or the *Pc68* gene were completely resistant in the laboratory to pathotypes of *P. coronata* f. sp. *avenae* isolated from different European countries (Sooväli and Koppel, 2003). Only *Pc38* showed a stable high level of resistance to natural oat crown rust in the four areas during the six years (Table 1 and 2). Although *Pc38* thus appears to have the best potential for use in breeding for crown rust resistance in Tunisia, high levels of virulence to *Pc38* have occurred in other countries. It is worth noting that a survey of *P. coronata* found that the *Pc38* line in Estonia was highly susceptible to

crown rust from 1996 to 2002 (Sooväli and Koppel, 2003). In Canada the frequency of virulence to *Pc38* increased from 59.2% in 1990 to 65.6% in 1991 (Chong and Seaman, 1993). It seems unlikely that a combination of the *Pc38*, *Pc39* and *Pc68* genes will provide a high level of durable protection from crown rust in Tunisia without the addition of some non-specific, partial resistance in commonly grown oat cultivars. Selection for adult plant, slow rusting resistance seems especially important for oat cultivars in areas of northern Tunisia with a high crown rust hazard.

It seems unlikely that populations of *P. coronata* on wild and commercial oats in northeastern or north-central Tunisia arise primarily from the aeciospores, because *R. lycioïdes*, the alternate host, occurs mainly in the mountains of northwestern Tunisia. Uredinispores produced locally on wild oat plants or volunteer oat plants are more likely to be the primary sources of infection, which may occur as early as November in northeastern and north-central Tunisia.

Early plantings of oats are highly receptive to crown rust, particularly in the coastal areas of Tunisia remote from the mountains where *R. lycioïdes* is common (Allagui *et al*., 2002). Consequently, volunteer oat plants and wild oat species may provide a green bridge for the survival of *P. coronata* between crop seasons in northeastern or north-central Tunisia. On the other hand, in northwestern Tunisia (Beja and Jendouba) the disease frequently appears on commercial oats in April and May at the time when the aeciospores are released (Hammami *et al*., 2006). The sexual stage of *P. coronata* may play a key role in crown rust epidemics in the northwestern Tunisia.

There was a high degree of similarity between the virulence profiles of *P. coronata* populations at Sedjinen and those at Alfereg (SI=4.51; Table 3). These two areas are close to the mountains where *R. lycioïdes* is common and where outbreaks of crown rust on oats are associated with the time of aeciospore release. Having a common source of initial inoculum may account for the similarities in virulence of Sedjinen and Alfereg. The levels of virulence of *P. coronata* populations in Ariana and Bourbia were very dissimilar to each other (SI=11.30; Table 3), and these populations were also dissimilar to the populations in northwestern Tunisia. As already mentioned, Ariana and Bourbia are distant from the natural range of *R. lycioides*. The most likely source of crown rust infection in Ariana and Bourbia are therefore urediniospores from infected wild and volunteer oat plants. The asexual nature of these populations and differences in the resistance genotypes of wild and volunteer oat plants that provide a green bridge between epidemics may have led to the difference in virulence patterns that was seen in northeastern and north-central Tunisia.

Although we believe that the sexual and asexual populations of *P. coronata* coexist in various parts of Tunisia, the occasional genetic exchange between populations cannot be ruled out. This possibility must be taken into account when it is considered whether combinations of effective *Pc*-genes in new oat cultivars can provide long-term protection from crown rust. For example, combinations of *Pc38*, *Pc39*, and *Pc68* may be difficult for asexual populations of *P. coronata* to overcome in a single step of three simultaneous mutations, but annual genetic recombination in sexual populations could facilitate the development of new races of *P. coronata* with a combined virulence that can overcome all three resistance genes. Even rare movements of virulent races of *P. coronata* from *R. lycioïdes* in northwestern Tunisia to northeastern or north-central Tunisia may initiate a rapid loss of effectiveness of the combined resistance of *Pc38*, *Pc39*, and *Pc68*. It would be helpful to have additional evidence about the frequency of genetic exchange, if any, between *P. coronata* populations in Tunisia. For example, comparisons of randomly amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP) markers between populations in Sedjnen, Afereg, Ariana, and Bourbia may provide a better understanding of whether populations of eastern and western Tunisia are genetically isolated.

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