J. Crop Prot. 2012, 1 (3): 189-199

Genetic diversity in different populations of citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in Tunisia, assessed by RAPD-PCR

Dhia Bouktila[1, 2*], Saïda Kharrat[3], Maha Mezghani-Khemakhem[1], Abderrahmane Jerraya[4] and Mohamed Makni[1]

1. Research Unit on Genomics of Crop Insect Pests (GIRC), Faculty of Sciences of Tunis, University of Tunis-El-Manar, Tunisia.
2. Higher Institute of Biotechnology of Béja, University of Jendouba, Tunisia.
3. Faculty of Sciences of Bizerte, University of Cartago, Tunisia.
4. Department of Entomology, National Institute of Agronomy, Tunis, Tunisia.

Abstract: The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is a major invasive pest of citrus in Tunisia. In order to help the implementation of an efficient integrated management strategy, it was essential to assess the genetic diversity and population structure of the pest. For this purpose, random-amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was applied, using eight oligo-nucleotide primers, to reveal genetic variability among eight populations of *P. citrella*, originating from the north, center and south of Tunisia. A total of 66 RAPD markers and 33 phenotypes were generated. Inter-population polymorphism was revealed, using the percentage of polymorphic markers (62.12 %), mean number of phenotypes generated per primer (4.125) and mean genetic distance (0.199). Hierarchical analysis, using the UPGMA method, indicated that the genetic variability was influenced by the regional distribution. This pattern of population clustering was supported by Principal Coordinate Analysis (PCO). Yet, a weak correlation (0.69) was revealed between genetic and geographic distances, suggesting that climatic contrariety between the north and south of Tunisia plays a major role in the differentiation of *P. citrella*, leading to a restriction of gene flow between populations. Results obtained in this work show clear genetic differences, which should be considered in the development of control strategies.

Keywords: Citrus, genetic diversity, pest management, *Phyllocnistis citrella*, RAPD-PCR.

Introduction

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a pest native to southern Asia (Heppner, 1995). Adult CLM is a very small, light-colored moth, less than 2 mm in length. Females lay their eggs singly on the underside of the newly emerging leaves near midribs or veins (Kharrat and Jerraya, 2005). Egg hatch takes 2 to 6 days, the average larval (4 instars) and pupal durations are 7-8 and 8-9 days, respectively, while the total life cycle from egg laying to adult emergence takes about 18 days (Ba-Angood, 1977, 1978). Multiple overlapping CLM generations per year are likely to occur (Xiao, 2009). Larvae feed by creating shallow tunnels, referred as mines, in young leaves of citrus trees. Mining the young foliage reduces growth rate and yield and the mines serve as foci for the establishment of the citrus canker

Handling Editor: Dr. Ehsan Rakhshani

* **Corresponding author**, e-mail: dhia_bouktila2000@yahoo.fr
Received: 8 May 2012; Accepted: 21 July 2012

[DOR: 20.1001.1.22519041.2012.1.3.10.5]

bacterium, *Pseudomonas citri* Hasses (Smith and Hoy, 1995; Gottwald *et al.*, 2001).

It has been demonstrated that *P. citrella* can develop a high degree of resistance to a broad range of insecticides (Villanueva-Jiménez and Hoy, 1988) making essential the development of alternative management methods, replacing the traditional chemical control. In this context, a differential susceptibility has been reported among citrus genotypes from India (Batra and Sandhu, 1983), Australia (Wilson, 1991), Spain (Jacas *et al.*, 1997) and Brazil (Santos *et al.*, 2011). The existence of antibiosis mechanisms of resistance has also been indicated in some studies (Batra and Sandhu, 1983; Jacas *et al.*, 1997), while in others, a modification in plant phenology leading to avoidance from *P. citrella*, has been described (Singh *et al.*, 1988; Padmanaban, 1994).

CLM has been reported in most parts of the world including Asia (Chiu, 1985; Uygun *et al.*, 1995), Australia (Wilson, 1991), Africa (Badawy, 1967; Berkani, 2003; Kheder *et al.*, 2002), the United States of America (Heppner, 1993, 1995; Heppner and Dixon, 1995), central America (Hoy and Jessey, 2004) and south America (Bermudez *et al.*, 2004). The insect has colonized citrus-growing areas in the Mediterranean Basin during the last decade of the 20th century (Urbaneja *et al.*, 2001). In Tunisia, CLM was detected for the first time in 1994, in the region of Tabarka (EPPO, 1998) and has been spreading to all regions, to constitute today a significant threat to citrus species, reaching an infestation rate of 100 % (Kheder *et al.*, 2002). Genetic monitoring of pest populations, including the assessment of genetic diversity, identification of diversification patterns, determination of migration history and pathways and characterization of virulent biotypes, plays an important role in the establishment of pest management strategies, as well as their optimization for a better efficiency and durability. The random-amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique has been used in several studies, to evaluate the genetic diversity of some major invasive species, such as the Mediterranean fruit fly, *Ceratitis capitata* (Wiedmann) (Haymer *et al.*, 1997), the potato whitefly, *Bemisia tabaci* (Gennadius) (Hasan, 2006) and the date palm root borer, *Oryctes agamemnon* Burmeister (Abdallah *et al.*, 2012). In the present study, we used the RAPD-PCR technique to analyze the genetic variability and population structure of *P. citrella* populations collected in different regions in Tunisia, in order to offer insights into the ecology of the species and provide practical information for optimizing crop protection programs.

Materials and Methods

Insect sampling

Sampling sites were eight orchards located in eight different regions in Tunisia (Figure 1). Sampling date and geographical location of each site are summarized in Table 1. Weather data of each sampling site were provided by Bioclimatology Laboratory at the *Institut National de Recherche en Génie Rural, Eaux et Forêts* (I. N. R. G. R. E. F., Tunisia) (Table 1). From each site, approximately 100 larvae of *P. citrella* were collected from various citrus trees (*Citrus* spp.). These larvae were placed in 96 % ethanol inside labelled tubes, in order to use them in molecular analysis.

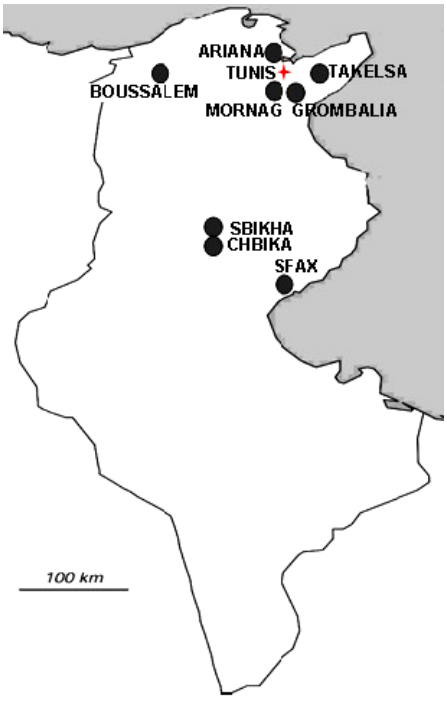

Figure 1 Map of Tunisia, showing the geographical location of *P. citrella* sampling sites (●).

[DOR: 20.1001.1.22519041.2012.1.3.10.5]

DNA extraction and RAPD-PCR amplification

From each *P. citrella* population, 10 CLM larvae were bulked together and used to extract genomic DNA. Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit A1120 (Promega, France), following the manufacturer instructions. RAPD-PCR reactions were performed in 25 µl reaction mix containing 50 ng (1 µl) of template genomic DNA, 1.75 units (0.35 µl) of Taq DNA Polymerase (GoTaq, Promega, France), 100 mM of each dNTP, 4 µM of a single 10-nucleotide primer, 5 µl 5X Taq Polymerase buffer and 0.5 µl of $MgCl_2$, supplied by the enzyme manufacturer. A set of eight primers supplied by Sigma-Aldrich (USA) was used for RAPD amplification (Table 2). Amplifications were performed in a thermal cycler Applied Biosystem 2720 programmed as follows: one cycle of 5 min at 94 °C, followed by 35 cycles of 1 min at 35 °C, 1 min at 95 °C and 1 min at 72 °C, followed by final extension step of 7 min at 72 °C. A negative control without DNA was added in each run to test for contamination. In order to avoid non reproducible markers, each experiment was replicated twice and only intense, reproducible fragments were considered for the statistical analysis. Amplification products were separated by electrophoresis on 1.5 % agarose gel, then visualized under UV light and photographed after staining in ethidium bromide. The molecular weight of each DNA band was estimated by comparing with a co-migrating 1 kb ladder (Promega, France).

Data analysis

Amplification patterns generated by each RAPD primer were transformed into binary data, where the presence of a marker was coded 1 and its absence 0. A RAPD fragment was considered as polymorphic once it was present in at least one bulked population and absent in the remaining ones. The percentage of polymorphic markers (% P) was calculated. The finalized fragment data from each primer were pooled to define binomial phenotypes. The number of phenotypes (P) generated by each primer was calculated. Using the program GENDIST of the PHYLIP software package version 3.68c (Felsenstein, 2008), a pairwise genetic distance matrix was constructed between the eight studied populations of *P. citrella*, based on the genetic distance of Nei and Li (1979). The Mantel test was applied to estimate the correlation between geographical and genetic distances, using the SPSS version 14.0 software (SPSS Inc., 2005). In order to illustrate the genetic relationships between the eight studied populations of CLM, the genetic distance matrix was submitted to cluster analysis by the Unweighted Paired Group Method for the Arithmetic Average (UPGMA) (Sneath and Sokal, 1973), by applying a 1000 pseudo-replicates bootstrap re-sampling, to assess the support for individual nodes. This analysis was performed using the program NEIGHBOR of the PHYLIP software package version 3.68c (Felsenstein, 2008). Finally, the genetic distance values were used as input data for two-dimensional principal coordinate analysis (2D PCO - Huff, 1997), in order to study the variation between *P. citrella* populations.

Results

RAPD-PCR amplification overview

Amplification of genomic DNA, obtained from eight studied *P. citrella* populations, generated reproducible and consistent amplification patterns. A total of 66 different markers were scored with the eight primers used, ranging from 150 to 1500 bp in size. The number of distinct markers observed for each primer ranged from 4 to 10 (Table 2).

Level of genetic diversity

The percentage of polymorphic markers varied between 25 % using OP-H01 and 87.50 % using OP-A02 (Table 2), indicating that primers differed in their efficiency to discriminate between the studied populations of CLM. The mean value of % P, once all primers were considered together, was 62.12 % (41/66),

[DOR: 20.1001.1.22519041.2012.1.3.10.5]

indicating a moderate polymorphism rate among populations. Thirty-three distinct phenotypes were generated using the eight primers, ranging between 2, with primers OP-D01/ OP-H01, and 8, with primer OP-A02, with an average number of 4.125 different phenotypes generated by each single primer (Table 2). The lowest genetic distance (0.0014) was found between Sfax and Chbika, whereas the highest genetic distance (0.5328) was between Takelsa and Sbikha. The remaining distances ranged between the values mentioned above, with an average of 0.199 between pairs of populations (Table 3).

Table 1 Sampling, geographical and meteorological data on the studied *P. citrella* populations, in Tunisia.

Site	Department	Geographical zone	Sampling date	Annual rainfall (mm)	No of days with rainfall / year
Takelsa 36° 47' N, 10° 37' E	Nabeul	North-East	19/06/2009	642	95
Grombalia 36° 36' N, 10° 29' E	Nabeul	North-East	18/06/2009	642	95
Mornag 36° 41' N, 10° 17' E	Grand Tunis	North-East	19/06/2009	615.5	100
Ariana 36° 49' N, 10° 9' E	Grand Tunis	North-East	11/06/2009	615.5	100
Boussalem 36° 37' N, 8° 58' E	Jendouba	North-West	19/06/2009	632,2	127
Sbikha 35° 53' N, 10° 2' E	Kairouan	Center	13/06/2009	337	58
Chbika 35° 37' N, 10° 2' E	Kairouan	Center	02/06/2009	337	58
Sfax 34° 44' N, 10° 45' E	Sfax	South	19/09/2009	287,6	41

Table 2 Nucleotide sequences, number of markers generated (N), size range (S), number (P) and percentage (% P) of polymorphic markers and number of phenotypes generated (PH), of eight RAPD-PCR primers used.

Primer code	Nucleotide sequence (5'→3')	N	S (bp)	P	%P	PH
OP-A01	CAGGCCCTTC	10	250-510	7	70.00	4
OP-A02	TGCCGAGCTG	8	340-1500	7	87.50	8
OP-A07	GAAACGGGTG	8	220-700	6	66.66	3
OP-D01	ACCGCGAAGG	7	340-800	2	28.57	2
OP-D02	GGACCCAACC	10	150-1000	6	60.00	5
OP-K02	GTCTCCGCAA	10	150-900	8	80.00	6
OP-I03	CAGAAGCCCA	9	150-500	4	44.44	3
OP-H01	GGTCGGAGAA	4	200-550	1	25.00	2
Total	-	66	150-1500	41	-	33
Mean	-	-	-	-	62.12	4.125

[DOR: 20.1001.1.22519041.2012.1.3.10.5]

Table 3 Pairwise genetic distances matrix between eight studied populations of *P. citrella*, in Tunisia.

	Takelsa	Grombalia	Mornag	Ariana	Boussalem	Sbikha	Chbika	Sfax
Takelsa	0.0000							
Grombalia	0.3023	0.0000						
Mornag	0.4613	0.1651	0.0000					
Ariana	0.3629	0.1911	0.2177	0.0000				
Boussalem	0.3947	0.1651	0.1911	0.0674	0.0000			
Sbikha	**0.5328**†	0.3321	0.3023	0.1651	0.1398	0.0000		
Chbika	0.4964	0.1911	0.2177	0.1911	0.2177	0.1651	0.0000	
Sfax	0.4966	0.1901	0.2171	0.1910	0.2167	0.1644	**0.0014**†	0.0000

† *The highest and lowest distances*

Population genetic structure

The dendrogram yielded by the UPGMA method (Figure 2) showed that *P. citrella* populations clustered into 2 distinct clades: a first clade (A), including populations from the north of Tunisia and a second clade (B), including populations of Sbikha and Chbika, both located in the center of Tunisia, as well as the southern population of Sfax. Clade A was subdivided into two sub-clades: the first one (A1) included the north-western population of Boussalem and the population of Ariana belonging to the department of Grand Tunis. The second sub-clade (A2) contained two populations from the north-east (Takelsa and Grombalia), as well as the population of Mornag belonging to Grand Tunis.

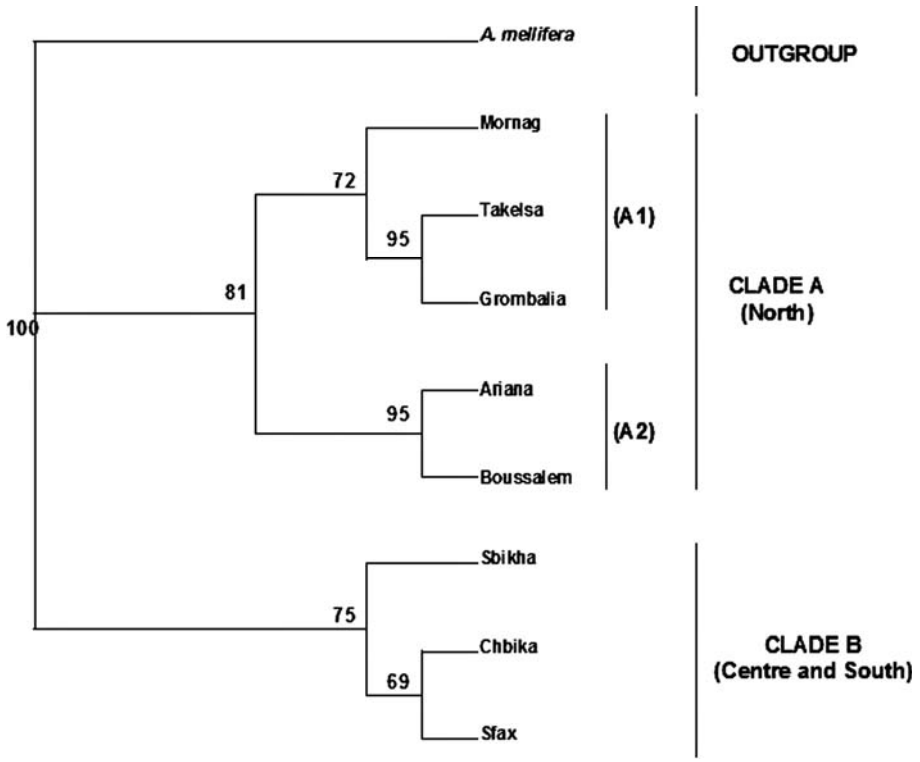

Figure 2 UPGMA dendrogram, showing genetic relationships between eight studied populations of *P. citrella*, from Tunisia. *Apis mellifera* (Hymenoptera: Apidae) was used as root. Bootstrap values, supporting each individual node, are shown.

[DOR: 20.1001.1.22519041.2012.1.3.10.5]

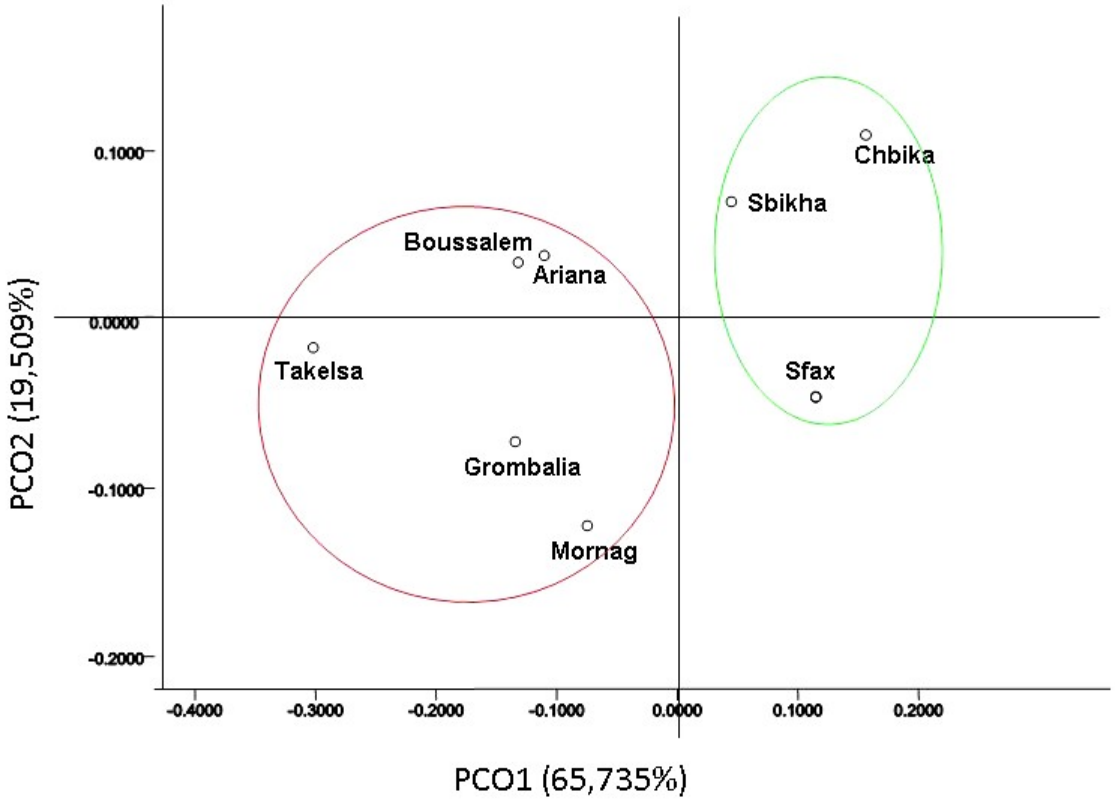

Figure 3 Two-dimensional principal coordinate analysis scatter plot showing patterns of diversity among eight *P. citrella* populations, studied based on 66 RAPD markers.

Axis 1 of the PCO plot (Figure 3) absorbed 65.735 % of the genetic variation and axis 2 absorbed 19.509 %. The plot was in agreement with the UPGMA dendrogram in that it revealed a regional differentiation of *P. citrella* populations. Two groups of populations were distinguished: a first group containing Takelsa, Grombalia, Mornag, Ariana and Boussalem populations located in the north; and a second group comprising 3 populations from the center (Sbikha and Chbika) and the south (Sfax).

Although clear geographic differentiation could be inferred from both UPGMA and PCO analyses, the Mantel test showed only a weak correlation (0.69) between genetic and geographic distances among *P. citrella* populations. Indeed, Ariana and Mornag populations were distant in genetic space (0.2177), although they are close in geographic space. In contrast, Ariana and Boussalem, which are separated by a large geographic distance, were close in genetic space (0.0674).

Discussion

The major aim of this study was to document the level and distribution of genetic diversity among Tunisian populations of *P. citrella*. Given the discrete nature of RAPD, it is probable that most of the revealed markers are amplified products of the less functional parts of the genome (Mamuris *et al.*, 2002). The percentage of polymorphic RAPD fragments (62.12 %), mean number of phenotypes

[DOR: 20.1001.1.22519041.2012.1.3.10.5]

generated per primer (4.125) and mean genetic distance (0.199) indicated a moderate among-population polymorphism. This fact was expected as DNA bulks were compared, which would probably have concealed DNA within-population polymorphisms, making only DNA fragments testifying a between-population polymorphism revealed. Therefore, the detected polymorphism would reflect only a portion of the expected full reservoir of genetic variability in *P. citrella*. James *et al*. (1999) reported that high genetic diversity characterizes usually insect species that reproduce sexually and have broad ecological niches and a wide geographical distribution. In addition, Nylin and Gotthard (1998) considered the length of generation time as a major factor in determining the amount of genetic diversity in insect populations, because it is directly related to the risk of death before the reproductive stage. CLM is characterized by a sexual reproduction, high number of generations per year and short period of sexuality (18 days). Besides, CLM has a broad ecological niche (several citrus species and cultivars can serve as hosts) and a wide geographical distribution (Kheder *et al.*, 2002; Kharrat and Jerraya, 2005), all over the territory of Tunisia. All these observations make *P. citrella* a good candidate for sexual recombination events between individuals within each population.

UPGMA and PCO analyses showed that the grouping of populations was related to a regional criterion, as northern populations (Mornag, Grombalia, Takelsa, Ariana and Boussalem) were clearly differentiated from central and southern populations (Sbikha, Chbika and Sfax). This regional differentiation indicates a restricted amount of gene flow occurring between populations from the north and those from the center and south of Tunisia. This observation might result from evolutionary changes due to genetic mutations occurring in populations subjected to a selection pressure exerted by climatic factors. This differentiation would have followed the recent invasion of citrus by CLM in Tunisia. Although populations from the north of Tunisia and those from the center and south were genetically differentiated, no strong correlation was revealed between the geographic and genetic distance matrices, as could be inferred from the Mantel test. Indeed, within the group comprising CLM populations from the north, the PCO and UPGMA analyses did not illustrate a strict correspondence with the geographic distribution of *P. citrella* populations. This result suggests that the contrasted climate between the north and the south of Tunisia has a strong selective effect, leading to a nearly independent mutation accumulation between populations in the two habitats. The meteorological characteristics of the sites where CLM samples were collected for this study (Table 1) tend to support this hypothesis. In fact, Takelsa, Grombalia, Ariana, Mornag and Boussalem are characterized by an annual rainfall over 600 mm, while this parameter does not exceed 400 mm in Sbikha, Chbika and Sfax in the south of Tunisia. In the same way, the number of days with rainfall per year seems much contrasted between the north, where it is around or above 100 days and the south where it is under 60 days. In a previous research on *P. citrella* in Tunisia, Kheder *et al.* (2002) found that the mortality rate of the pest was influenced by climatic conditions such as the high heat of the summer and the low temperatures. In the case of many agricultural insect pests, a genetic structuring in relation with climatic scale has been reported, such as Leite *et al.* (2004), who found that the intensity of *B. tabaci* attack on tomato, in Brazil, was positively correlated with mean temperature but they did not observe any significant effect of rainfall. A genetic pattern related to micro-climate has also been reported in *Sitobion avenae* Fabricius (De Barro *et al.*, 1995; Dedryver *et al.*, 2008), *Myzus persicae* Sulzer (Guillemaut *et al.*, 2003) and *Metopolphium dirhodum* Walker (Nicol *et al.*, 1997). Although RAPD markers, used in this study, are discrete ones, the genetic structure revealed could be potentially linked to biological traits (i. e. mortality rate, virulence intensity), playing a

[DOR: 20.1001.1.22519041.2012.1.3.10.5]

role in the pest-host interaction processes occurring during infestation.

The result of this study was useful to reveal a significant potential for adaptation behind the clear genetic differentiation of *P. citrella* in Tunisia, allowing genotypes of this invasive insect species to quickly adapt to contrasted environmental conditions. This clear genetic differentiation should be considered in the development of control strategies. Therefore, because of the high economic impact of CLM on citrus production, genetic monitoring efforts should be continued, through more extensive sampling and use of additional molecular markers, in parallel with eradication measures and restrictions on the export of fruits produced in infested regions.

Acknowledgements

The authors gratefully recognize funding support from the Tunisian Ministry of Higher Education and Scientific Research.

References

Abdallah, Z., Mezghani-Khemakhem, M., Bouktila, D., Makni, H. and Makni M. 2012. Genetic diversity of an invasive pest (*Oryctes agamemnon* Burmeister, *Coleoptera*: Scarabaeidae) of date palm in Tunisia, inferred from random amplified polymorphic DNA (RAPD) markers. African Journal of Agricultural Research, 7: 1170–1176.

Ba-Angood, S. A. S. 1977. A contribution to the biology and occurrence of the Citrus leafminer, *Phyllocnistis citrella* Staint. (Gracilariidae, Lepid.) in the Sudan. Zeitschrift für Angewandte Entomologie, 83: 106–111.

Ba-Angood, S. A. S. 1978. On the biology and food preference of the citrus leafminer, *Phyllocnistis citrella* Stainton (Gracillariidae), in PDR of Yemen. Zeitschrift fur Angewandte Entomologie, 86: 53–57.

Badawy, A. 1967. The morphology and biology of *Phyllocnistis citrella* Staint., a citrus leaf miner in the Sudan. Bulletin of the Entomological Society of Egypt, 51: 95–103.

Batra, R. C. and Sandhu, G. S. 1983. Screening of citrus germplasm for citrus leafminer in the Punjab. Journal of Research Punjab Agricultural University, 18: 221–223.

Berkani, A. 2003. Morphometric study of the preimaginal stages of *Phyllocnistis citrella* Stainton (Lepidoptera, Gracillariidae) in Algeria. Fruits, 58: 83–88.

Bermudez, E. C., Martinez, N. B., Graziano, J. V., Bernal, H. C. A. and Paniagua, A. H. 2004. *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its parasitoids in citrus in Ecuador. Florida Entomologist, 87: 10–17.

Chiu, S. C. 1985. Biological control of citrus pests in Taiwan. Taiwan Agricultural Research Institute Special Report 19:1–8.

De Barro, P. J., Sherratt, T. N., Brookes, C. P., David, O. and Maclean, N. 1995. Spatial and temporal genetic variation in British field populations of the grain aphid *Sitobion avenae* (Hemiptera: Aphididae) studied using RAPD-PCR. Proceedings of the Royal Society of London, Series B, 262: 321–327.

Dedryver, C. A., Gallic, J. F., Haak, L., Halkett, F., Outerman, Y. and Simon, J. C. 2008. Seasonal and annual genotypic variation and the effect of climate on cereal aphid *Sitobion avenae* in Northern France. Bulletin of Entomological Research, 98: 159–168.

EPPO, 1998. Situation of *Phyllocnistis citrella* in the European, Mediterranean and Near East regions. European and Mediterranean Plant Protection Organization (EPPO) Reporting Sevice, 3: 13-15. http://archives.eppo.int/EPPOReporting/1998/Rse-9803.pdf

Felsenstein, J. 2008. PHYLIP (Phylogeny Inference Package) Version 3.68. Department of Genetics, University of Washington, Seattle (WA).

Gottwald, T. R., Hughes, G., Graham, J. H., Sun X. and Riley T. 2001. The citrus canker epidemic in Florida: The scientific basis of regulatory eradication policy for an invasive species. Phytopathology, 91: 30–34.

[DOR: 20.1001.1.22519041.2012.1.3.10.5]

Guillemaut, T., Mieuzet, L. and Simon, J. C. 2003. Spatial and temporal genetic variability in French populations of peach potato aphid, *Myzus persicae*. Heredity, 91: 143–152.

Hasan, S. H. 2006. Survey of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) Biotypes in Jordan using RAPD markers. Journal of Entomology, 3: 290–297.

Haymer, D. S., He, M. and McInnis, D. O. 1997. Genetic marker analysis of spatial and temporal relationships among existing populations and new infestations of the Mediterranean fruit fly (*Ceratitis capitata*). Heredity, 79: 302–309.

Heppner, J. B. 1993. Citrus leafminer *Phyllocnistis citrella* in Florida (Lepidoptera: Gracillariidae: Phyllocnituinae). Tropical Lepidoptera, 4: 49–64.

Heppner, J. B. 1995. Citrus leafminer (Lepidoptera: Gracillariidae) on fruit in Florida. Florida Entomologist, 78: 183–186.

Heppner, J. B. and Dixon, W. N. 1995. Potential spread of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae: Phyllocnistinae) in the United States. American Entomologist, 41: 110–113.

Hoy, M. A. and Jessey, C. 2004. *Ageniaspis citricola* (Hymenoptera: Encyrtidae) established in Bermuda. Florida Entomologist, 87: 229–230.

Huff, D. R., 1997. RAPD characterization of heterogeneous perennial ryegrass cultivars. Crop Science, 37: 557–564.

Jacas, J. A., Garrido, A., Margaix, C., Forner, J., Alcaide, A. and Pina, J. 1997. Screening of different citrus rootstocks and citrus-related species for resistance to *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Crop Protection, 16: 701–705.

James, T. Y., Porter, D., Hamrick, J. L. and Vilgalys, R. 1999. Evidence for limited intercontinental gene flow in the cosmopolitan mushroom, *Schizophyllum commune*. Evolution, 53: 1665–1667.

Kharrat, S. and Jerraya, A. 2005. Lien entre la préférence d'oviposition et la performance subséquente des larves chez la mineuse des agrumes *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). Phytoprotection, 86: 25–29.

Kheder, S. B., Jerraya, A., Jrad, F. and Fezzani, M. 2002. Étude de la mineuse des agrumes *Phyllocnistis citrella* Stainton (Lep. Gracillariidae) dans la région du Cap Bon (Tunisie). Fruits, 57: 29-42.

Leite, G. L. D., Picanco, M., Jham, G. N. and Moreira, M. D. 2004. Natural factors influencing whitefly attack in tomato. Arquivos do Instituto Biologico (Sao Paulo), 71: 245–248.

Mamuris, Z., Sfougaris, A. I., Stamatis, C. and Suchentrunk, F. 2002. Assessment of Genetic structure of Greek Brown Hare (*Lepus europaeus*) populations based on variation in random amplified polymorphic DNA (RAPD). Biochemical Genetics, 40: 323–338.

Nei, M. and Li, W. H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of USA, 76: 5269–5273.

Nicol, D., Armstrong, K. F., Wratten, S. D., Cameron, C. M., Frampton, C. M. and Fenton, C. M. B. 1997. Genetic variation in an introduced aphid pest *Metopolphium dirhodum* in New Zealand and relations to individuals from Europe. Molecular Ecology, 6: 255–265.

Nylin, S. and Gotthard, K. 1998. Plasticity in life-history traits. Annual Review of Entomology, 43: 63–83.

Padmanaban, B. 1994. Screening of citrus germplasm for controlling citrus leaf-miner (*Phyllocnistis citrella*) (Lepidoptera: Phyllocnistidae). Indian Journal of Agricultural Sciences, 64: 723–726.

Santos, M. S., Vendramim, J. D., Lourençao, A. L., Pitta, R. M. and Martinus, E. S. 2011. Resistance of Citrus Genotypes to *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). Neotropical Entomology, 40: 489–494.

Singh, S. P., Rao, N. S., Kumar, K. K. and Bhumannavar, B. S. 1988. Field screening of citrus germplasm against the citrus

[DOR: 20.1001.1.22519041.2012.1.3.10.5]

leafminer *Phyllocnistis citrella* Stainton. Indian Journal of Entomology, 50: 69–75.

Smith, J. M. and Hoy, M. A. 1995. Rearing methods for *Ageniaspis citricola* (Hymenoptera: Encyrtidae) and *Cirrospilus quadristriatus* (Hymenoptera: Eulophidae) released in a classical biological control program for the citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Florida Entomologist, 78: 600–608.

Sneath, P. H. A. and Sokal, R. R. 1973. Numerical taxonomy. Freeman, San Francisco.

SPSS Inc., 2005. SPSS Base 14.0 User's Guide, USA.

Urbaneja, A., Llacer, E., Garrido, A. and Jacas, J. A. 2001. Effect of temperature on the life history of *Cirrospilus* sp. near *lyncus* (Hymenoptera: Eulophidae), a parasitoid of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Biological Control, 21: 293–299.

Uygun, N., Karaca, I., Aytas, M., Yumruktepe, R., Yigit, A., Ulusoy, M. R., Kersting, U., Tekeli, N. Z. and Canhilal, R. 1995. A serious citrus pest: Citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillaridae). Turkiye Entomoloji Dergisi (Turkish Journal of Entomology), 19: 247–252.

Villanueva-Jiménez, J. A. and Hoy, M. A. 1988. Constraints on developing an integrated pest management program for citrus leafminer (Lepidoptera: Gracillariidae) in Florida nurseries. Horticulture Technology, 8: 332–345.

Wilson, C. G. 1991. Notes on *Phyllocnistis citrella* Stainton (Lepidoptera: Phyllocnistidae) attacking four citrus varieties in Darwin. Journal of the Australian Entomological Society, 30: 77–78.

Xiao, Y. 2009. Biology, ecology and management of key pests of Satsuma citrus in Alabama. PhD dissertation, Auburn University, Auburn, Alabama, USA 177 pp.

[DOR: 20.1001.1.22519041.2012.1.3.10.5]

ارزیابی تنوع ژنتیکی جمعیت‌های پروانه مینوز مرکبات، *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) در تونس با استفاده از روش RAPD-PCR

دیا بوکتیلا[۱و۲*]، سیدا خرّاط[۳]، ماها مزقانی- خماخم[۱]، عبدالرحمان جرایا[۴] و محمد مکنی[۱]

۱- بخش تحقیقات ژنتیک آفات گیاهان زراعی (GIRC)، دانشکده علوم تونیس، دانشگاه المنار تونیس، تونس
۲- مؤسسه عالی تحقیقات بیوتکنولوژی بَجا، دانشگاه جندوبا، تونس
۳- دانشکده علوم، بیزرت، دانشگاه کارتاکو، تونس
۴- گروه حشره‌شناسی، مرکز ملی علوم زراعی، تونیس، تونس
* پست الکترونیکی نویسنده مسئول مکاتبه: dhia_bouktila2000@yahoo.fr
دریافت: ۱۹ اردیبهشت ۱۳۹۱؛ پذیرش: ۳۱ تیر ۱۳۹۱

چکیده: پروانه مینوز مرکبات،*Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) مهمترین آفت وارداتی مرکبات در کشور تونس می‌باشد. ارزیابی تنوع ژنتیکی و ساختار جمعیتی آفت برای اجرای مؤثر استراتژی مدیریت تلفیقی آن ضروری است. به‌همین منظور، تکنیک RAPD-PCR مبتنی بر هشت پرایمر الیگونوکلئوتید، برای تعیین تنوع ژنتیکی هشت جمعیت مختلف از *P. citrella* جمع‌آوری شده از مناطق شمالی، مرکزی و جنوبی تونس استفاده شد. در مجموع ۶۶ مارکر رپید و ۳۳ فنوتیپ مشخص گردید. پلی‌مرفیسم بین جمعیتی براساس درصد مارکرهای پلی‌مرفیک (۶۲/۱۲ ٪)، میانگین تعداد فنوتیپ‌های مشخص شده براساس هر پرایمر (۴/۱۲۵) و میانگین فاصله ژنتیکی (۰/۱۹۹) تعیین شد. تحلیل سلسله مراتبی مبتنی بر روش UPGMA نشان داد که تنوع ژنتیکی متأثر از الگوی پراکنش منطقه‌ای است. صحت این الگو با تحلیل PCO نیز اثبات گردید. در عین حال، همبستگی ضعیف (۰/۶۹) بین دو پارامتر فاصله ژنتیکی و فاصله جغرافیایی نیز نشان‌دهنده این موضوع است که اختلاف شرایط کلیماتیک شمال و جنوب کشور تونس نقش مهمی در واگرایی جمعیت‌های *P. citrella* دارد که منجر به محدود شدن تبادل ژنتیکی بین جمعیت‌ها شده است. نتایج به‌دست آمده از این تحقیق نشان‌دهنده وجود تفاوت آشکار ژنتیکی است که بایستی در زمان برنامه‌ریزی برای کنترل آفت در نظر گرفته شود.

واژگان کلیدی: مرکبات، تنوع ژنتیکی، مدیریت آفات، *Phyllocnistis citrella*، RAPD-PCR

[DOR: 20.1001.1.22519041.2012.1.3.10.5]