Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

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Egypt. Acad. J. Biolog. Sci., 9(3): 9-20(2017)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
www.eajbs.eg.net

Distribution and identification of potato cyst nematodes from Ain Defla region, Algeria

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ARTICLE INFO

Article History
Received: 25/9/2017
Accepted: 10/11/2017

Keywords:
PCN
Distribution
Identification
Ain Defla
Algeria

ABSTRACT

Potato cyst nematodes PCNs are the most economically damaging pest of potato crop worldwide. During 2013, a survey was carried out in Ain Defla region of Algeria. 81 soil samples collected from potato fields of 14 localities were submitted to nematological analysis which revealed the presence of these nematodes in 22.22% of the prospected fields. Sixteen PCN populations from five localities were characterized by combination of features the perineal regions of cysts and those of second stage juveniles. The morphological identification has been confirmed by the analysis of the ITS-RFLP profiles, sequencing and phylogenetic analysis of the ITS region. The results revealed that the two species *Globodera rostochiensis* and *G. pallida* are present in this region occurring separately or in mixed populations. However, we noted a dominance of *G. pallida* since only 12.25% of the populations have been identified like *G. rostochiensis* whereas 31.5% were *G. pallida* and 56, 25% of the populations were constituted of a mixture of the two species and among these mixed populations, 77.77% presented a dominance of *G. pallida.* The predominance of *G. pallida* has been noted in the sites of Ain Defla, El Amra, Mekhatria and Arib. *G. rostochiensis* was dominant in Rouina. Intraspecific variation was noted between populations of *G. rostochiensis* and *G. pallida*. Because of the high divergence among Algerian populations of *G. pallida* and *G. rostochiensis* it can be assumed that they were multi-introduced in Algeria. The most divergent population of *G. pallida*, that formed a well separated group with some populations from Chile and Peru, suggests a later or independent introduction of this population in Algeria.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of major crops contributing to the world food requirement (Karam *et al*., 2009). In Algeria, potato is the second most important crop after cereals and is becoming an increasingly source of food (Yahiaoui *et al.*, 2003). However, there are a number of biotic (fungal and bacterial disease and pests) and abiotic (environmental conditions) constraints that threaten potato production. One of the main biotic constraints to potato production is potato cyst nematode PCN. The golden nematode *Globodera rostochiensis* and pale nematode *Globodera pallida* (Stone, 1973) cause qualitative and qualitative losses to potato crops as they justify their classification as quarantine pests in several countries (Chauvin et *al.,* 2008; Niere and Unger, 2012).

In Algeria, PCNs were detected for the first time in 1953 following the introducing of potato seed of British origin at the end of the Second World War (Frézal, 1954). By 1961, the infested area had increased greatly in 33 townships around Algiers (Scotto La Massese 1961). Thereafter, the plant protection services and some unpublished works signaled their dissemination in several potato growing areas which the important are Ain Defla, Tipaza, Chlef, Mascara and Sétif.

An integrated approach to PCN management is mainly based on crop rotation and use of resistant varieties. Monitoring is essential to detect the fields contaminated by these parasites to take the measures that limit their dissemination in other regions. However, an exact identification of the present species in the infested sites is important to develop control strategies because the two PCN species can react differently to control measures (Madani *et al.*, 2010).

In Ain Defla which is a province (wilaya) located in Midwest of Algeria, the main activity revolves around agriculture and potato is a very important crop and can be cultivated throughout the year and covered nearly half (50%) of the total agricultural production. So, Knowledge about the repartition of potato cyst nematodes and species identification is necessary to initiate measures of control. Therefore, the aims of this study were to: 1) determine the occurrence and distribution of potato cyst nematodes in potato growing areas of Ain Defla 2) Identification of species which occur in the different localities of this region.

MATERIALS AND METHODS

Study area:

The study area is located in the Midwest of Algeria. It is situated to 140 km of the Algiers capital (36° 15' 55'' N, 1° 58' 13'' E).This area has a typical Mediterranean semi-arid with long hot summer and short warm, winter. The considerable difference between the monthly maximum temperatures ranging from (46°C) in July to (0°C) in January reflects a continental character. In this region, fine textured soils are predominant (80 %) and more than 45 % of them are represented by clay soils. The arable land is around 235,611 ha representing 51.85 % of the total area, with 731 million of kg of potatoes the region ranks second in Algeria in term of potato production (DSA, Ain Defla 2013).

The survey is carried out in fourteen selected localities with potato vocation which are among the most producers of this vegetable in this province.

Sampling and extraction of nematodes:

Soil sampling was conducted during 2013. About 2 kg of soil was taken from each field at the depth of 30 cm and consisting of several elementary samples of 100 g collected from across the two diagonals of the field in the rhyzosphere of potato. Their number depends on the surface of the treated field. It is about 20 subsamples per ha. In the laboratory, the soil samples were dried (Nakachia and Jacqemont, 1971) and weighed. Then cysts were extracted from 1kg of soil by Fenwick's apparatus based on the waterline (Fenwick 1940). Empty and full cysts are recovered separately in Petri dishes using a fine brush under a binocular microscope.

Species identification:

The species identification was conducted at the Nematology Laboratory *Istituto per la Protezione Sostenibile delle Piante, Bari, Consiglio Nazionale delle Richerche (CNR).* Sixteen populations of PCN belonging to five sites were collected: four from El Amra EAG1, EAG2, EAG3 and EAG4) four from Rouina (RG1, RG2, RG3 and RG4), Six from Ain Defla (ADG1, ADG2, ADG3, ADG4, ADG5 and ADG6), one from Arib (ARG) and one from Mekhatria (MEG). All sixteen PCN populations are identified morphologically but only six populations were identified by molecular techniques.

Morphomological identification:

The species identification was based on slides of permanent mounts according to Hooper (1970). Juveniles were fixed in formaldehyde à 4% + 1% propionic acid and processed to glycerin. Cysts were similarly fixed in 40% lactic acid, 30% glycerol and 30% distilled water. The specimens were examined by optic microscope and measurements were made. Specimens were identified with recent taxonomic keys and a compendium for identification of *Globodera spp.* (Golden, 1986; Baldwin and Mundo-Ocampo, 1991).

Molecular identification:

We used PCR-RFLP technique to confirm species identity of six populations ADG3, EAG2 ARG, MEG, RG2 and RG3. The digestion was made with the restriction enzymes *Alu*I et *Rsa*I (Skantar et *al.*, 2007). PCR products of the ITS region were purified for sequencing using the NucleoSpin® Extract II (Macherey-Nagel). Purified DNA fragments were cloned and send for sequencing, in both directions, at MWG-Eurofin in Germany. A BLAST (Basic Local Alignment Search Tool) was performed in order to confirm their nematode origins and species (Altschul *et al*., 1997). The newly obtained sequences for ITS region and were aligned using ClustalW (Larkin *et al*., 2007) with default parameters with the corresponding published gene sequences of *Globodera* species. Phylogenetic trees were performed with Neighbour-Joining (NJ), Minimum Evolution (ME), Maximum Likelihood (ML) and Maximum Parsimony (MP) methods using MEGA version 6 software Tamura *et al*., 2013). No significant conflict in branching order and support level among methods is observed, only ML tree is shown.

RESULTS

Infestation frequency:

Filled cysts of *Globodera* spp. were detected in six of the fourteen surveyed localities (El Amra, Rouina, Ain Defla, Mekhatria, Arib and Attaf). The frequency of infestation varied considerably between localities. The highest frequency was recorded at Rouina 83.33% where five out of the six prospected plots proved infested. In El Amra, Ain Defla and Attaf, it was 66.67%. At Mekhatria, 33.33% of analyzed samples were infested. Only a sample of the six (16, 67%) analyzed at Arib was infested. All plots located in the other localities were free as no full cysts are detected. 18 plots out of 81 surveyed, 22.22% were infested with PCN (Table 1).

Table 1: Infestation frequency in the surveyed localities.

Commune	Number of analyzed samples	Number of uninfested samples	Number of infested samples	% of uninfested samples	% of infested samples
El Amra	6	2	4	33.33	66.67
Rouina	6	1	5	16.67	83.33
Ain Defla	6	2	4	33.33	66.67
Attaf	3	1	2	33.33	66.67
Mekhatria	6	4	2	66.67	33.33
Bourached	6	6	0	100	0
Bordj Amir Khaled	6	6	0	100	0
Bir Ouled Khalifa	6	6	0	100	0
Arib	6	5	1	83.33	16.67
Djendel	6	6	0	100	0
Ain Soltane	6	6	0	100	0
Zeddine	6	6	0	100	0
El Abadia	6	6	0	100	0
Djelida	6	6	0	100	0
Total	**81**	**63**	**18**	**77.78**	**22.22**

Species identification

Sixteen PCN populations collected from five localities in Ain Defla (El Amra,

Rouina, Ain Defla, Mekhatria, Arib) were identified by combining perineal region and juveniles characters. The results show the presence of the two species *G. rostochiensis* and *G. pallida.* They can occur separately or on in mixed populations. In the locality of Ain Defla, 66, 66 % of collected populations were identified as *G. pallida* and 33.34% are mixed populations. 75% of populations of El Amra were *G. pallida* and the other 25% were mixture of *G. pallida* and *G. rostochiensis*. In the region of Rouina, 50% of identified populations were *G. rostochiensis* and 50% were mixed population. The two populations of Mekhatria and Arib were mixture of the two species (Table 2 and Fig.1).

Table 2: Nematode species identified in the different localities of Ain Defla region

Identified species/ Population	*G. rostochiensis*	*G. pallida*	Mixed population (dominance of *G. pallida*)	Mixed population (dominance of *G. rostochiensis*)
Ain Defla	-	ADG1, ADG2 ADG3, ADG4	ADG5, ADG6	-
El Amra	-	EAG2	EAG1, EAG3, EAG4	-
Rouina	RG2, RG3	-		RG1,RG4
Mekhatria	-	-	MEG	-
Arib	-	-	ARG	-

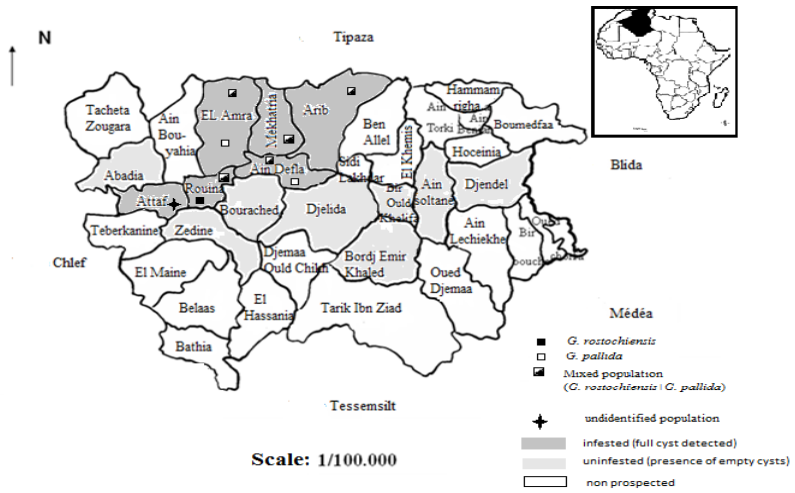

Fig. 1: Location of the collection sites of PCN in Ain Defla-Algeria.

The results show high frequency of mixed populations (56.25% of identified PCN populations). *G. pallida* was identified as the dominant species in the surveyed region: 31.5% of the characterized PCN populations were pure *G. pallida* and this species was also prevalent in 77.78% (seven of the nine) of the mixed populations. Only 12.25% of the sixteen analyzed PCN populations were pure *G. rostochiensis*. This species was dominant, only in 22.22% (two of the nine) of mixed populations (Fig. 2).

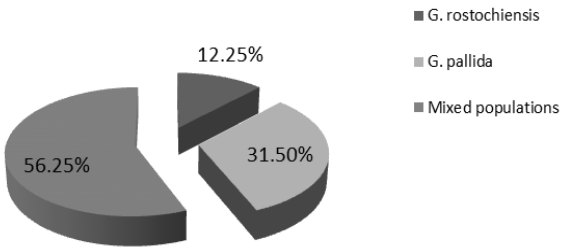

Fig. 2: Frequency of potato cyst nematodes species in studied region.

Morphological identification:

The cysts morphometric data and those of juveniles were within the ranges reported in the literature for both species with some variation noted between populations. Clear differences were observed between pure *G. rostochiensis* populations and pure *G. pallida* populations (Figs. 3 and 4). The mean of number of cuticular ridges of the five populations of *G. pallida* was 12.8 and it was 19.9 for *G. rostochiensis*. The mean of Granek's ratio was lower for *G. pallida* (2.4) than *G. rostochiensis* populations (4.1). In mixed populations; heterogeneity was noted by analyzing the cyst perineal regions and juveniles characters. For stylet length, the means were 23.3 µm for *G. pallida* and 21.2 µm for *G. rostochiensis*. Stylet knobs shape was also different between the *G. pallida* populations (anteriorly pointed for *G. pallida* and rounded for *G. rostochiensis*).

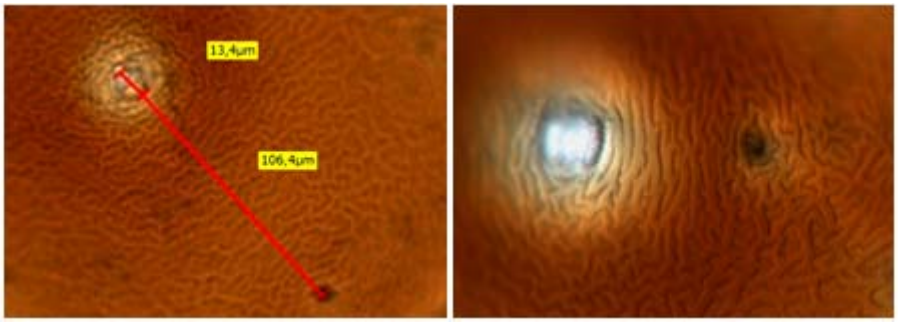

Fig. 3: Microscopic views of perineal patterns of 1) *Globodera rostochiensis* and 2) *Globodera pallida*

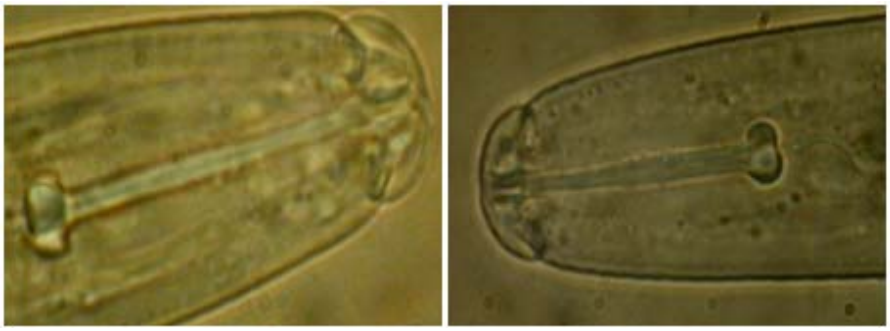

Fig. 4: Microscopic views of anterior part of juveniles of 1) *Globodera rostochiensis* and 2) *Globodera pallida*

Molecular identification

PCR-RFLP diagnostics

Amplification of the ITS region of *G. pallida* and *G. rostochiensis* yielded single fragments of 1188 bp, 1190 bp respectively. Diagnostic PCR-ITS-RFLP profiles for *G. pallida* and *G. rostochiensis* from Ain Defla region of Algeria are given in (Fig. 5). The two diagnostic enzymes produced RFLP patterns that allowed discriminating the two *Globodera* species from Algeria, even for mixed populations.

The *Alu* I (lanes 1, 2, 5 and 7; fragment sizes 505 and 383 bp) digestion of Algerian *G. pallida* populations showed identical restriction profile among geographical populations and were identical to those of *G. pallida* from Perù, York and Idaho (USA) (Skantar et *al.*, 2007; Subbotin et *al.*, 2011).

The enzyme *Rsa* I (lanes 8-9, 12 and 14; fragment sizes 587 and 385 bp) produced in *G. pallida* populations from Ain Defla and El Amra a restriction profile (lanes 8 and 9) identical to that of *G. pallida* populations from Europe, Asia, North and South America and Oceania, while populations from Arib and Mekatria showed a polymorphism at *Rsa* I site that result in missing restriction sites (lanes 12 and 14).

The *Alu* I and *Rsa* I profiles for Algerian *G. rostochiensis* populations were identical each other but different from those of *G. rostochiensis* determined so far.

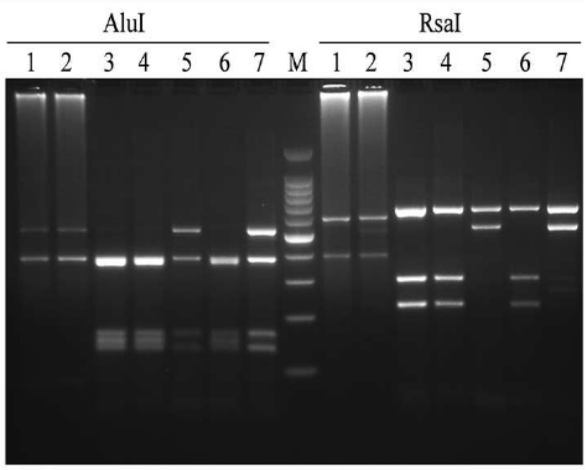

Fig. 5: PCR-RFLP profiles for *Globodera pallida* and *G. rostochiensis* populations from Algeria digested by using the *Alu* I and *Rsa* I enzymes. M: 100 bp ladder DNA; lane 1: *G. pallida* from Ain Defla; lane 2: *G. pallida* from El Amra; lane 3: G. *rostochiensis* from Rouina 1; lane 4: *G. rostochiensis* from Rouina 2; lane 5: *G. pallida* from Arib; lane 6: *G. rostochiensis* from Arib; lane 7: *G. pallida* from Mekhatria

Phylogenetic analysis:

Sequence alignment included 64 sequences, 11 (6 for *G. pallida* e 5 for *G. rostochiensis)* of which were newly obtained in this study. Intraspecific variations among Algerian populations were as follows: *G. pallida* (6 sequences) 7-22 nucleotides; *G. rostochiensis* (5 sequences) 11-20 nucleotides. For *G. pallida* the most variable population was *G. pallida* from Mekhatria and for *G. rostochiensis* from Rouina RG3.

Phylogenetic relationships of *G. pallida* and *G. rostochiensis* from Algeria were inferred from analyses of the ITS sequences of the closest species of cyst nematodes by using ML method. Sequence alignment of the ITS regions included 64 sequences, 11 (6 for *G. pallida* e 5 for *G. rostochiensis*) of which were newly obtained in this study. *Globodera artemisiae* and *G. capensis* were used as outgroups for the ITS dataset. The ML tree of the ITS (Fig. 6) is reported and consisted of three groupings: 1) all *G. pallida* and *G. mexicana* isolates, 2) all *G. rostochiensis* and *G. tabacum* isolates, 3) *G. artemisiae* and *G. capensis*. Group 1 contained four subgroupings: I) *G. pallida* sequences from Algeria (Ain Defla, Arib and El Amra) that fitted into a highly supported clade (92% support) including all sequences from Europe, Peru, Australia and Idaho (USA); II) *G. mexicana* group as sister species of subgroup I; III) *G. pallida* sequences from Mekhatria (Algeria), Peru, and Chile; IV) *G. pallida* from Peru and South Africa isolates at basal position (86% support) of all *G. pallida* isolates used in this analysis. Group II consisted of four subgroupings: V) *G. pallida* from Argentina confirming that this isolate could represent a hybrid or a naturally occurring variant closely related to *G. rostochiensis*; VI) *G. tabacum* closely related to *G. rostochiensis* (95% support); VII) *G. rostochiensis* from Bolivia grouped as sister species of all *G. rostochiensis* (86% support); VIII) *G. rostochiensis* isolates from Algeria, Europe and Canada.

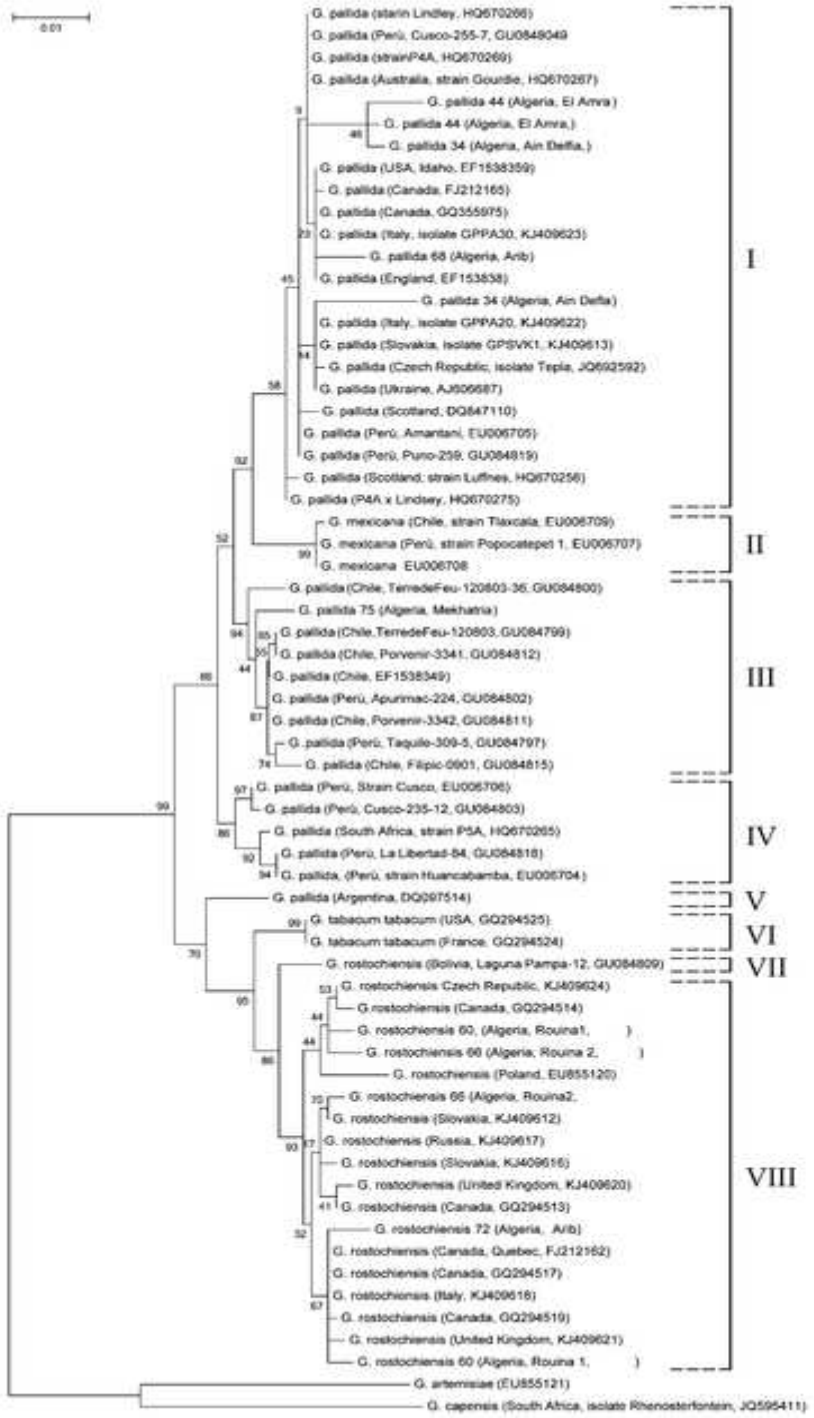

Fig. 6: Phylogenetic tree of ITS containing region describing the evolutionary relationships among different geographical populations of *Globodera pallida* and *G. rostochiensis* using Maximum Likely (ML) method. Branch lengths are proportional to the distances as derived from the distance matrix obtained using the GTR method with the invariant site plus gamma options. Numbers at nodes indicate bootstrap values.

DISCUSSION

The aim of the present study was to determine the current prevalence, incidence and distribution of potato cyst nematodes in Ain Defla region, which is one of the potato growing areas of Algeria.

Our investigations have shown that in Ain Defla region, potato cyst nematodes are confined to some localities because the parasite was detected in viable cysts in only six of the fourteen studied localities namely Rouina, El Amra, Ain Defla, Mekhatria, Arib and Attaf. 18 plots of 81 surveyed (22.22%) were infested with PCN. In 63 of the 81 plots studied, no eggs or larvae are found in the collected cysts. The absence of full cysts in some samples does not necessarily mean that these localities are free of these parasites.

The data revealed that the two PCN species are present in the surveyed region, *G. rostochiensis* and *G. pallida* can occur together or separately in cultivated sites of potato. *G. pallida* is dominant in the localities of Ain Defla and El Amra, But *G. rostochiensis* is dominant in the locality of Rouina. Only one population was studied In Mekhatria and Arib and the results showed that they were mixed populations with dominance of *G. pallida.*

The results show high frequency of mixed populations (56.25% of the identified PCN populations). The occurrence of mixed populations of *G. pallida* and *G. rostochiensis* in the same field was also reported in South America (Evans *et al.*, 1975), in New Zeland (Marshall, 1993) in Northern Irland (Turner, 1996), in England and Wales (Ibrahim *et al*., 2001) and recently in Czech Republic (Douda *et al*., 2014). The incidence of mixed populations of two species complicates control measures using resistant cultivars to only one species.

In all mixed populations, there was predominance of either of the two species. In the majority of the mixed populations (these collected in Ain Defla, El Amra, Arib and Mekhatria), there was dominance of *G. pallida*. However, for the two mixed populations from Rouina, we noticed a dominance of *G. rostochiensis*. The dominant species in mixed PCN populations is possibly the result of inter-specific competition. Den Nijs (1992) reported that an interaction exists between these two species when they occur together. For these two closely related species which have the same feeding sites on the same host, interactions are generally assumed to be mutually antagonistic because the nematodes compete for the available feeding site.

The different responses of the two species to soil type, cultural practices (cultivars, crop rotation, irrigation) and also the initial nematode density could created the situation that in the localities of Ain Defla, El Amra, Mekhatria and Arib, *G. pallida* is the most common species, whereas in Rouina *G. rostochiensis* is dominant. In all five localities, potato is generally, grown in rotation with cereals and the most cultivated variety is *Spunta* which is susceptible cultivar to the two species (Greco *et al*., 2007), Neverthless, farmers, sometimes, introduce others potato varieties in rotation such as *Bartina*, *kondor*, *Désirée* and *Atlas* which can have different effect on the reproduction of the two species. No information is available about resistance of potato cultivars grown under Algerian conditions to potato cyst nematodes. Thus, experiments must be carried out in order to evaluate resistance level of the most important potato cultivars grown in Algeria. Also, the dominance of *G. pallida* in the region of Ain Defla can be explained with the early harvesting of potato crops made by farmers of this region, in order to get the highest market prices. This practice may favor *G. pallida* as females of since species were reported to develop slightly faster than those of *G. rostochiensis* (Webley and Jones, 1981).

G. pallida was identified as the dominant species in Ain Defla region of Algeria, which is characterized by hot semi-

arid Mediterranean climate with temperatures in the higher 20 °C in the high summer. However, Hlaoua *et al.* (2008) reported the dominance of *G. pallida* in the region of Bizerte of Tunisia which is characterized by cold temperate climate and the prevalence of *G. rostochiensis* in the regions of Sousse and Montzair which are characterized by hotter climate.

Digestion of PCR-ITS products with the enzymes Alu I and Rsa I clearly discriminated Algerian *G. pallida* and *G. rostochiensis* populations. *G. pallida* from Mekhatria and Arib localities showed identical restriction profile by using *Rsa* I but different from 1 those of *G. pallida* from Ain Defla and El Amra regions. Heterogeneity in ITS of Peruvian *G. pallida* populations was already reported by RFLP analysis and several restriction enzymes discriminate European and Peruvian populations (Grenier *et al*., 2001). *Globodera rostochiensis* populations from Algeria showed identical restriction profiles but different from other populations of *G. rostochiensis* reported in literature (Subbotin *et al*., 2000).

In the present study the newly obtained sequences of *G. pallida* from Algeria are distributed within two clades: *G. pallida* of Ain Defla, Arib and El Amra clustered in the subgroup I. The G. *pallida* isolate from Mekhatria resulting the most divergent isolate of *G. pallida* from Algeria, grouped instead in the subgroup III together with isolates of *G. pallida* from different areas of Chile and Peru. This supports the hypothesis of multi-introductions of *G. pallida* into Algeria from South America (Perù and Chile) and from Europe and North America. The phylogenetic analysis revealed that Mekhatria population could represent a genetically intermediate population. Although ITS sequences diversity for *G. rostochiensis* populations, from Algeria and from all over the world, was slightly less than that of *G. pallida*. In the phylogenetic tree all geographical populations of *G. rostochiensis* formed a supported cluster (with 93 % support) even some relationships among *G. rostochiensis* sequences were unresolved. It seems that the presence of *G. rostochiensis* in Algeria is not the result of direct import of infected potatoes from Latin America, but it is more likely that a country from Europe or Canada was the "transition host" as also found in Tunisia by Hlaoua *et al.* (2008).

This study has allowed determining the geographical distribution, the genetic variation and the origin of *Globodera* species from Ain Defla region of Algeria. The correct and rapid identification of PCN is essential for their control. This can be achieved by the combination of morphological criteria and molecular techniques. Throw this approach, we can identify the PCN species found in the studied region also we showed that the two species of PCN *G. rostochiensis* and *G. pallida* can occur either separately or in mixed populations in a same site. Other surveys should be done to complete the information on the distributions of PCNs in this area and in other areas of Algeria.

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RABIC SUMMARY

توزيع نيماتودا البطاطا المتحوصلة من منطقة عين الدفلى للجزائر

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نيماتودا البطاطا المتحوصلة تعد أكثر الآفات التي تسبب أضرارا اقتصادية بمحاصيل البطاطا في جميع أنحاء العالم. خلال عام ٢٠١٣، أجريت دراسة في منطقة عين الدفلى في الجزائر. تم تحليل ٨١ عينة تربة تم جمعها من حقول البطاطا من ١٤ ضاحية. كشفت هذه الدراسة وجود هذه النيماتودا في ٢٢،٢٢٪ من الحقول المراقبة. تم تشخيص ستة عشر سلالة من خمس ضواحي بفضل الجمع بين خصائص مناطق العجان للحويصلات وتلك الخاصة بيرقات الطور الثاني. وقد تم تأكيد التشخيص المورفولوجي بتحليل نتائج ITS-RFLP،التسلسل وتحليل النشوء والتطور في منطقة ITS .أظهرت النتائج أن كلا النوعين *Globodera pallida* و *Globodera rostochiensis* متواجدين في هذه المنطقة منفصلين أو في سلالات مختلطة إما إلا انه لوحظ هيمنة *G. pallida* حيث تم تحديد ١٢.٢٥٪ من السلالات G. *rostochiensis* في حين أن ٣١.٥٪ كانت *G. pallida*و ٥٦.٢٥٪ من السلالات تشكلت من خليط من النوعين، و من بين السلالات المختلطة، ٧٧،٧٧٪ أظهرت هيمنة *G. pallida* . لوحظت غلبة *G. pallida* في المواقع التالية : عين الدفلى، العامرة، مخاترية وعريب أما G. *rostochiensis* فكان مهيمنا في الروينة . أظهرت هذه الدراسة وجود اختلاف جيني ضمن نوعي بين *G. rostochiensis* و *G. pallida* سلالات وبسبب الاختلاف الكبير بين سلالات *G. pallida* و *G. rostochiensis* يمكن افتراض أنها كانت متعددة الدخول إلى الجزائر. بالنسبة للسلالة الأكثر تباينا التي شكلت مجموعة منفصلة جدا مع بعض سلالات من التشيلي والبيرو يفترض أن دخولها إلى الجزائر كان متأخرا أو مستقلا.

الكلمات الرئيسية : نيماتودا البطاطا المتحوصلة ، توزيع، تشخيص ، عين الدفلى، الجزائر.