Journal of Entomology

ISSN 1812-5670

Academic Journals Inc.

www.academicjournals.com

Journal of Entomology 8 (4): 341-352, 2011
ISSN 1812-5670 / DOI: 10.3923/je.2011.341.352

Idiosomal Setae and Genetic Analysis in *Oligonychus punicae* and *Oligonychus biharensis* (Acari, Tetranychidae) Populations from State of Lara, Venezuela

[1]Carlos Vásquez, [1]Gerardo Castillo, [1]Martha Davila and [2]Alexander Hernandez
[1]Departamento de Ciencias Biológicas, Universidad Centroccidental Lisandro Alvarado, Decanato de Agronomía, Cabudare, Estado Lara, Venezuela
[2]Universidad Centrocidental Lisandro Alvarado, Postgrado de Agronomia, Cabudare, Estado Lara, Venezuela

Corresponding Author: Carlos Vásquez, Departamento de Ciencias Biológicas, Universidad Centroccidental Lisandro Alvarado, Decanato de Agronomía, Cabudare, Estado Lara, Venezuela

ABSTRACT

In this study, variations in idiosomal setae length and genetic similarity using RAPD-PCR technique were evaluated in *Oligonychus punicae* (Hirst) or *Oligonychus biharensis* (Hirst) (Acari, Tetranychidae) females collected on grapevines or mango trees growing in Tarabana and El Tocuyo counties, Lara state, Venezuela. Idiosomal seta analysis (v_2, sc_1, sc_2, c_1, c_2, c_3, d_1, d_2, e_1, e_2, f_1, f_2, h_3) showed significant differences in sc_1 and sc_2 in *O. punicae* from both localities, meanwhile in *O. biharensis* setae v_2, sc_1, c_1, d_1, e_1 and f_1 were found to be different in same localities. The Component Principal Analysis (ACP) on idiosomal setae showed that *O. punicae* population from Tarabana (TARVID) is similar to those from El Tocuyo (TOCVID). Meanwhile, *O. biharensis* populations exhibit higher variability in idiosomal setae length than *O. punicae* populations. Genetic analysis, DNA amplification by RAPD yielded 218 bands, being 175 (80%) polymorphic and 43 (20%) monomorphic. Higher number of bands was obtained with primer OPB10, suggesting it would be able to detect higher polymorphism in individuals studied.

Key words: Fruit-trees, genetic, grapevine, morphometrym, tetranychid

INTRODUCTION

Tetranychidae includes about 1,200 described species and some of them have been economic importance pests (Bolland *et al*., 1998), mainly species belonging to genus *Tetranychus* and *Oligonychus* (Zhang, 2003). In *Oligonychus*, 213 species have been reported feeding mainly on trees, shrubs and perennial grasses (Bolland *et al*., 1998).

The Avocado Brown Mite (ABM), *Oligonychus punicae* (Hirst), is a main pest in several avocado cultivars (*Persea americana* L.) in most of the producer areas in California, where high population levels have been observed causing defoliation (Cerna *et al*., 2009). *O. punicae* has been reported on more than 20 host plant species, including *Mangifera indica* L., *Musa sapientum* L. and *Punica granatum* L. (Ochoa *et al*., 1994; Bolland *et al*., 1998) and it is widely distributed in Neotropical countries like Colombia, Costa Rica, Cuba, El Salvador, Honduras, Mexico, Nicaragua, Panama and Venezuela (Bolland *et al*., 1998). In Venezuela, the ABM has been reported on *Musa* spp. and causing considerable damage on leaves from various *Vitis vinifera* L. cultivars (Vasquez *et al*., 2008).

On the other hand, the Cassava Red Mite (CRM), *Oligonychus biharensis* (Hirst) has been observed on guava in Malaysia (Gould and Raga, 2002) and more recently CRM has been reported occurring on *Clitoria* sp. in Venezuela (Vasquez *et al*., 2009). In China, it is a major pest on litchi, primarily during summer and autumn seasons (Chen *et al*., 2005). According to Bolland *et al*. (1998), *O. biharensis* occurs largely in Asian countries but it has been registered in Brazil and Mexico, however, so far it has not been recorded in Venezuela, so that this work constitutes the first record in the country.

Frequently, as response to environmental variations some morphological variations are verified in Tetranychid species as response to environmental variations ranging from phenotypic plasticity to speciation (Meyers and Bull, 2002) with genetic polymorphism and biotypes and semi-isolated races formation in intermediate position (Magalhaes *et al*., 2007). Furthermore, population density and geographical distances are considered as main factors affecting genetic flux in natural populations of *Tetranychus urticae* Koch (Tsagkarakou *et al*., 1997; Carbonelle *et al*., 2007). Additionally, changes of host plant could originate new species reproductively isolated from sympatric progenitors (Tsagkarakou *et al*., 1999; Magalhaes *et al*., 2007) which reproductive incompatibilities are considered as genetic divergences evidence (Navajas *et al*., 1994; Tsagkarakou *et al*., 1997).

Several statistical tools have been used to distinguish between species and intraespecific variations in different arthropod taxa. Moder *et al*. (2007) based on morphometric data sets from the ant genera *Cardiocondyla*, *Lasius* and *Tetramorium* used a discriminant analysis procedure in species distinction for finding the optimal character combination. Gettinger and Owen (2000) suggested three distinct host-associated *Androlaelaps rotundus* populations in Paraguay, by using a multivariate analysis of morphometric data. Furthermore, although the value of each band may measured by RAPD not have the same weight in an evolutionary context (Hance *et al*., 1998), technique has shown to be an valuable tool to better understand genome relationship of related plant species (Patra *et al*., 2011; Lakshmi *et al*., 2008) but also it has been widely used for microorganisms (Cumagun *et al*., 2007) and invertebrates (Hlaoua *et al*., 2008), including predatory and phytophagous mite species (Rodrigues *et al*., 2004; Yli-Mattila *et al*., 2000).

Despite the importance of both species of Tetranychidae worldwide, information about chaetotaxic length and genetic variation is not available in Venezuela, thus we intended to evaluate them in *O. punicae* and *O. biharensis* as effect of host plant and geographic distribution in state of Lara, Venezuela.

MATERIALS AND METHODS

Collection and identification of mite populations: *O. punicae* from *V. vinifera* and *O. biharensis* from *M. indica* were collected in Tarabana County (Palavecino municipality, 522 masl, 10°01'10" N and 69°16'55" W) and El Tocuyo County (Moran Municipality, 604 m, 9°47'55" N and 69°49'38" W), respectively in state of Lara, Venezuela from May 2007-December, 2008. Leaves showing damage symptoms produced by Tetranychidae were randomly collected from each host plant species and location. Damage was evidenced by yellowish spots on adaxial leaf surface (Jeppson *et al*., 1975). Subsequently, samples were placed in plastic bags and taken to the laboratorio de Zoología Agrícola, Decanato de Agronomia, Universidad Centroccidental Lisandro Alvarado, Venezuela. Each sample was labeled with data of location, host plant and collection date.

At the laboratory, females or males from both localities and host plants were mounted using Hoyer's medium (Krantz, 1978). Each slide was labeled, oven-dried at 44°C during 3-5 days and sealed. Species were identified using taxonomical key provided by Pritchard and Baker (1955)

and by comparison with the aedeagus morphology (Ochoa *et al.*, 1994). Once species were confirmed, females and males were reared in rearing units according to Helle and Overmeer (1985).

Seta analysis: Dorsal setae (v_2 = external ventrals; sc_1 = 1st scapulars; sc_2 = 2nd scapulars; c_1 = 1st dorsocentrals; c_2 = 1st dorsolaterals; d_1 = 2nd dorsocentrals; d_2 = 2nd dorsolaterals; e_1 = 3rd dorsocentrals; e_2 = 3rd dorsolaterals; f_1 = inner sacrals; f_2 = outer sacrals; h_3 = anterior para-anals) of *O. punicae* and *O. biharensis* females were measured as illustrated by Zhang (2003). Forty females were examined for each location and host plant species under a contrast-phase microscope magnification (Zeiss DM 1000). Data were subjected to a Principal Component Analysis (PCA) using the program NTSYS-PC version 1.7 (Sneath and Sokal, 1973). After PCA, variables resulting in significant differences were subjected to variance analysis using SAS JMP 5.0.1 (SASJMP, 2003) to determine morphological variations in populations.

Molecular studies: DNA extraction: Twenty females of each species/location were used for DNA extraction at Laboratorio de Virologia, Postgrado de Fitopatologia, Decanato de Agronomia, Universidad Centroccidental Lisandro Alvarado. DNA was extracted following Cenis (1993).

DNA amplification: Each single DNA was estimated in 10 ng by comparison methods and then amplified by Polymerase Chain Reaction (PCR) technique following Osakabe *et al.* (2000). Primers used were OPA01, OPA03, OPB10 and OPB13 (2ng) (Operon Technologies Co, from series A and B) in 1.5 μL Buffer (25 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 8.3), desoxinucleotids (dNTPs) (10 mM), Taq polymerase (5 μL^{-1}) and genomic mites DNA.

PCR was performed in a thermocycler (Perkin Elmer Gene Amp PCR System 2400) under following conditions: Pre-denaturing at 93°C for 1 min, followed by 45 denaturing cycles at 92°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 1 min, post-extension at 72°C for 5 min and preservation at 4°C.

Electrophoresis: Nine microliter of DNA from each sampled female was separated by electrophoresis on 1.5% agarose gel and run for 1.25 h at 80 V in a 1X TAE buffer (Tris base 40 mM; Acetato de Sodio 5 mM; EDTA 7, 7 mM, pH 8). Gels were stained with 1% ethidium bromide (BrEth) for 10 min. A 100 pb-pair of base titer was used. Finally, gels were photographed on an UV transilluminator using a Polaroid camera.

Statistical analysis: Data set were subjected to Principal Component Analysis (PCA) using NTSYS-PC version 2.1 (Rohlf, 1992). Afterward, variance analysis was performed to those variables resulting significant using SAS JMP 5.0.1 (SASJMP, 2003).

RESULTS

Seta analysis in *O. punicae* and *O. biharensis* populations: Both *O. biharensis* populations exhibited greater variability in the idiosomal setae length when compared to *O. punicae* populations. *O. biharensis* exhibited higher number of setae (v_2, sc_1, c_1, d_1, e_1 and f_1) showed to be different ($p<0.05$), whereas in *O. punicae* females only differences in sc_1 and sc_2 length of mites collected in Tarabana (TAR) and El Tocuyo (TOC) were found (Table 1). Thus, *O. punicae* populations collected from Tarabana (TARVID) and El Tocuyo (TOCVID) showed to be similar, since there is a tendency to remain in a single group (Fig. 1).

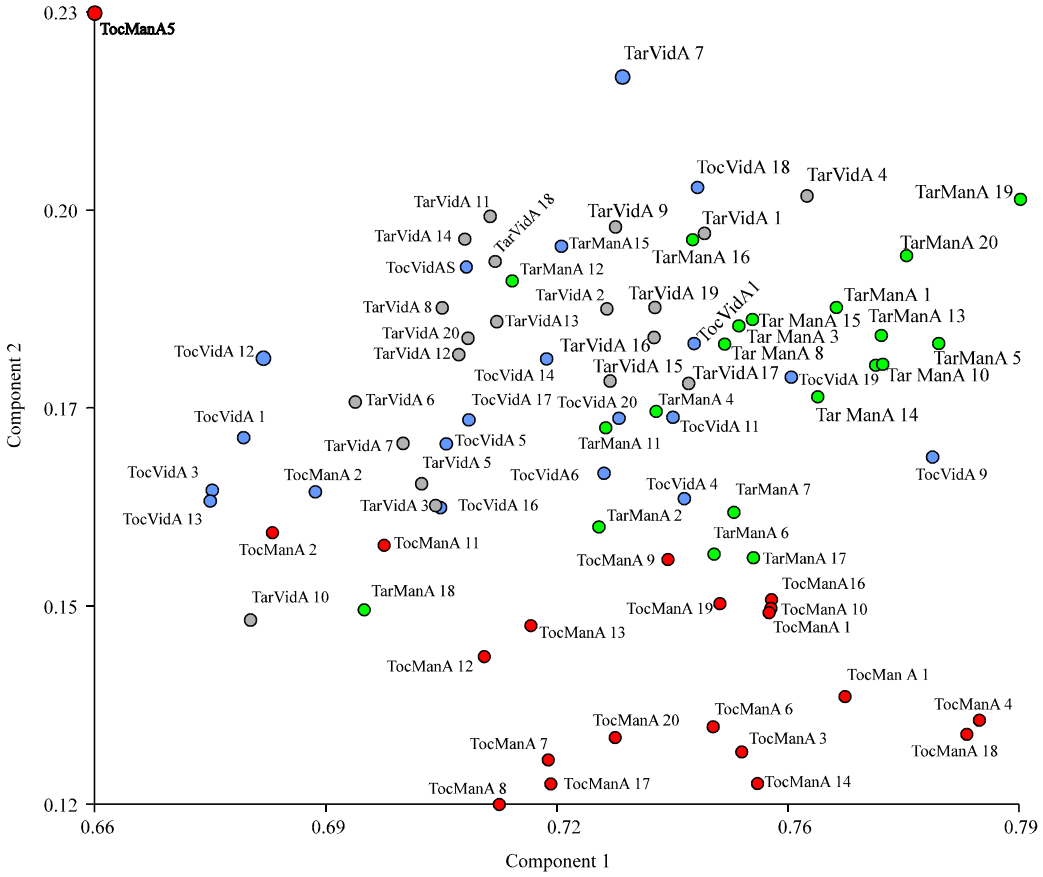

Fig. 1: Clustering of setae data from *O. punicae* (V = vid) and *O. biharensis* (M = mango) populations collected in Tarabana (Ta) and El Tocuyo (To) according to PCA

Table 1: Idiosomal dorsal setae mean length (µm) of *O. punicae* and *O. biharensis* from grape and mango trees collected in two localities (Tarabana (TAR) and El Tocuyo (TOC)) from Lara state, Venezuela

	O. punicae (grape)		*O. biharensis* (mango)	
Setae	Tarabana (TAR)	El Tocuyo (TOC)	Tarabana (TAR)	El Tocuyo (TOC)
v_2	0.63±0.0294^a	0.60±0.1312^a	0.65±0.0340^a	0.61±0.0363^b
sc_1	0.95±0.0237^a	0.92±0.0261^b	0.94±0.0440^a	0.86±0.0261^b
c_1	0.82±0.0319^a	0.80±0.0645^a	0.82±0.0433^a	0.76±0.0477^b
d_1	0.79±0.0486^a	0.78±0.0437^a	0.82±0.0358^a	0.77±0.0397^b
e_1	0.71±0.0630^a	0.75±0.0489^a	0.78±0.0397^a	0.73±0.0382^b
f_1	0.70±0.0261^a	0.70±0.0560^a	0.72±0.0366^a	0.68±0.0638^b
sc_2	0.78±0.0405^b	0.80±0.0405^a	0.76±0.0522^a	0.76±0.0419^a
c_2	0.79±0.0342^a	0.76±0.0779^a	0.84±0.0419^a	0.82±0.0698^a
d_2	0.83±0.0261^a	0.83±0.0437^a	0.88±0.0324^a	0.91±0.0599^a
e_2	0.79±0.0371^a	0.81±0.0433^a	0.85±0.0300^a	0.86±0.0658^a
f_2	0.53±0.0624^a	0.55±0.0558^a	0.58±0.0313^a	0.58±0.0358^a
h_3	0.80±0.0322^a	0.80±0.0383^a	0.82±0.0427^a	0.85±0.0809^a

Values are as Mean±SD, Values in a column followed by same letter did not show significant differences, according to t-student test (p>0.05)

Table 2: Principal Components Analysis (PCA) for idiosomal seta characterization

Component	Eigenvalue	Percentage	Accumulated
1	3.249627	27.0802	27.080222
2	2.653917	22.116	49.196233
3	1.304193	10.8683	60.064544
4	0.916178	7.6348	67.699355
5	0.842863	7.0239	74.723166
6	0.694512	5.7876	80.510777
7	0.593734	4.9478	85.458588
8	0.553547	4.6129	90.071499
9	0.354286	2.9524	93.02381010
10	0.310543	2.5879	95.61171111
11	0.293136	2.4428	98.05451212
12	0.233464	1.9455	>100%

Table 3: Component weights of used characters in setae characterization

Characters	Component C1	Component C2	Component C3	Component C4
v_2	0.4001	0.1442	0.4560	0.4504
sc_1	0.1674	0.8356	0.2154	-0.0139
c_1	0.3554	0.7053	0.0995	0.1020
d_1	0.4627	0.6659	-0.2314	-0.1310
e_1	0.5466	0.3993	-0.4620	-0.2543
f_1	0.4918	0.0963	0.2868	0.2874
sc_2	0.4045	0.0744	0.5413	-0.6619
c_2	0.6824	-0.1817	0.1154	0.2236
d_2	0.6465	-0.4278	-0.2917	0.0139
e_2	0.6614	-0.5187	-0.1005	-0.0634
f_2	0.6337	0.0085	-0.4154	0.1322
h_3	0.5424	-0.5786	0.3416	-0.1687

v_2 = External ventrals; sc_1 = 1st scapulars; c_1 = 1st dorsocentrals; d_1 = 2nd dorsocentrals; e_1 = 3rd dorsocentrals; f_1 = inner sacrals; sc_2 = 2nd scapulars; c_2 = 1st dorsolaterals; d_2 = 2nd dorsolaterals; e_2 = 3rd dorsolaterals; f_2 = outer sacrals; h_3 = anterior para-anals

According to the Principal Component Analysis (PCA), about 50% of variability in populations was accounted for the first two components (Table 2) and c_2, d_2, e_2 and f_2 setae showed more weight in component 1, whereas sc_1, c_1 and d_1 obtained more weight in component 2 (Table 3).

RAPD analysis: DNA amplification by RAPD yielded 218 bands, being 175 (80%) polymorphic and 43 (20%) monomorphic. Greater number of bands was obtained with primer OPB10, suggesting it would be able to detect higher polymorphism in individuals studied (Table 4).

Similarly, out of 121 total bands obtained in locality TAR, higher number of bands was obtained with primer OPB10 (37), being 31 bands polymorphic and 6 bands monomorphic. When primer OPB13 was used, 33 total bands were produced, all of them being polymorphic. Lower numbers of bands were observed by using primers OPA01 and OPA03 (Table 2). Primer OPB10 separated larger number of bands in populations from locality TOC, followed by OPA01 and OPA03 with 24 bands each and OPB13 with 19 bands (Table 5). No difference in number of polymorphic bands was observed in both locations (TAR = 90 bands and TOC = 85).

When number of DNA bands for mite species were analyzed, larger number was observed in *O. biharensis* (from Mango), showing 113 bands when primer OPB10 (37 total bands) was used,

Table 4: Number of bands of DNA in *O. punicae* and *O. biharensis* using different primers

	Primers				
Primers	OPA01	OPA03	OPB10	OPB13	Total
NMB	15	14	13	1	43
NPB	30	40	54	51	175
NTB	45	54	67	52	218

NMB = Number of monophormic bands; NPB = Number of polymorphic bands; NTB = Number of total bands. OPA01 = 5´-CAGGCCCTTC-3`; OPA03 = 5´-AGTCAGCCAC-3`; OPB10 = 5`-CTGCTGGGAC-3`; OPB13 = 5`-TTCCCCCGCT-3´

Table 5: Number of bands of DNA in *O. punicae* or *O. biharensis* according to localities

	Localities									
	TAR					TOC				
Primer	OPA01	OPA03	OPB10	OPB13	Total	OPA01	OPA03	OPB10	OPB13	Total
NMB	13	12	6	0	31	2	2	7	1	12
NPB	8	18	31	33	90	22	22	23	18	85
NTB	21	30	37	33	121	24	24	30	19	97

NMB = Number of monophormic bands, NPB = Number of polymorphic bands, NTB = Number of total bands, TAR = Tarabana County; TOC = El Tocuyo County

Table 6: Number of bands of DNA in *O. punicae* or *O. biharensis* according to host plant

	Host plant									
	Grape					Mango				
Primer	OPA01	OPA03	OPB10	OPB13	Total	OPA01	OPA03	OPB10	OPB13	Total
NMB	4	5	7	1	17	11	9	6	0	26
NPB	17	24	23	24	88	13	16	31	27	87
NTB	21	29	30	25	105	24	25	37	27	113

NMB = Number of monophormic bands, NPB = Number of polymorphic bands; NTB = Number of total bands, grape (*O. punicae*) and mango (*O. biharensis*)

followed by OPB13 (27), OPA03 (25) and OPA01 (24). Similarly, the highest number of polymorphic bands was obtained with OPB10, followed by OPB13 in samples collected from grapevine, being 105 bands similar to those in mango (Table 6).

On the other hand, when number of bands by crop was analyzed in TAR, higher number was obtained with primers OPA03 and OPB10 in grapevine, while in mango samples, higher number was obtained with OPB10 and OPB13 (Table 7). Furthermore, higher number of polymorphic bands was observed in samples collected on grape (55 polymorphic bands) compared to 35 bands in samples from mango.

Conversely, in samples from TOC, higher number of total and polymorphic bands was observed in samples from mango with primers OPB10 and OPB13 (18 and 11, respectively) (Table 7).

Genetic variation in *O. punicae* and *O. biharensis*: Higher variability was observed in *O. punicae* populations collected in TOC (33%) and TAR (28%), meanwhile in *O. biharensis*, variation was 21 and 12% in TOC and TAR, respectively. Similarity percentage between populations was 43, 37, 32 and 73% for TOCVID, TARVID, TOCMAN and TARMAN, respectively (Fig. 2).

Table 7: Number of bands of DNA from *O. punicae* or *O. biharensis* by host plant from localities Tarabana (TAR) and El Tocuyo (TOC)

	Grape					Mango				
Primer	OPA01	OPA03	OPB10	OPB13	Total	OPA01	OPA03	OPB10	OPB13	Total
Tarabana										
NMB	3	3	0	0	6	10	9	6	0	25
NPB	5	15	18	17	55	3	3	13	16	35
NTB	8	18	18	17	61	13	12	19	16	60
El Tocuyo										
NMB	1	2	7	1	11	1	0	0	0	1
NPB	12	9	5	7	33	10	13	18	11	52
NTB	13	11	12	8	44	11	13	18	11	53

NMB = Number of monophormic bands; NPB = Number of polymorphic bands; NTB = Number of total bands; grape (*O. punicae*) and mango (*O. biharensis*)

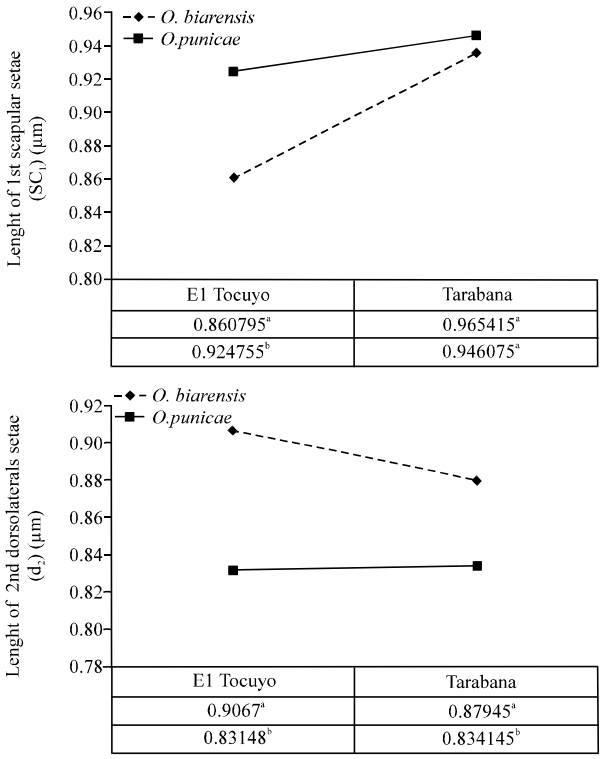

Fig. 2: Mean test for idiosomal setae sc_1 (top) and d_2 (bottom) in *O. punicae* and *O. biharensis* females collected in El Tocuyo and Tarabana. Mean values followed by the same letter are not significantly different according to least square mean test

O. biharensis populations showed tendency to be more separated than *O. punicae* populations (Fig. 3). In addition, based on RAPD analysis which accounted for 36% of variability, similarity coefficients for TOCVID, TOCMAN, TARVID and TARMAN were 0.56, 0.40, 0.47 and 0.78, respectively (Table 6). Thus, *O. biharensis* population from El Tocuyo showed higher similarity to *O. punicae* population from Tarabana.

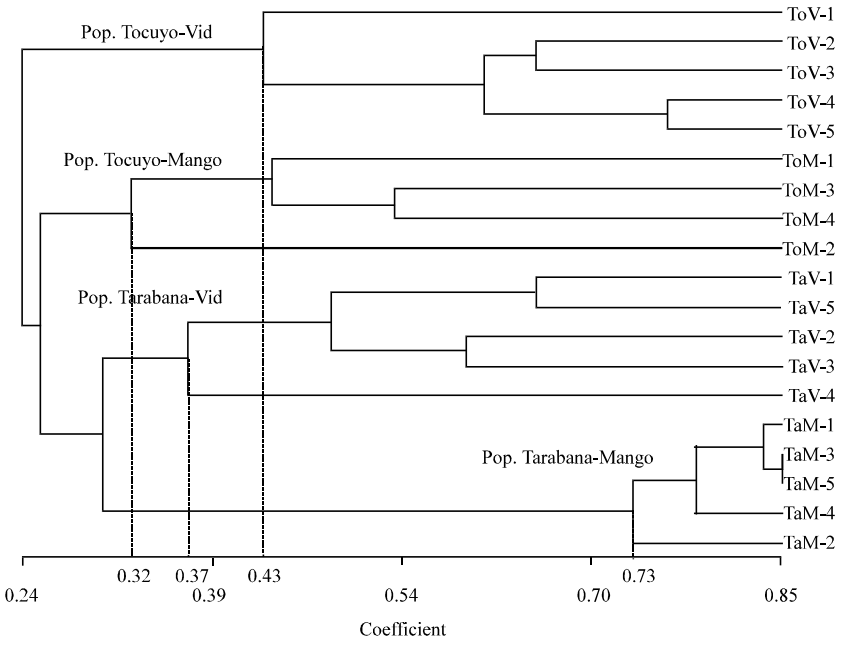

Fig. 3: Dendrogram obtained by UPGMA algoritm of Jaccard's index between *O. punicae* (VID) and *O. biharensis* (MAN) collected in Tarabana (TAR) and El Tocuyo (TOC)

DISCUSSION

The Principal Component Analysis (PCA) of the idiosomal seta data showed that subset of characters could be useful for species characterization. On the other hand, setae in *O. biharensis* populations from Tarabana (TARMAN) and El Tocuyo (TOCMAN) seem to be more discriminating, thus indicating that populations are separated by location possibly due to environmental conditions in each area (Carbonelle *et al.*, 2007).

Although studied species are morphologically related, seta analysis by PCA could be useful in species discrimination due to, on one hand, analysis separated both species, being *O. punicae* in the upper left and *O. biharensis* in the lower right and on the other hand, disaggregated *O. biharensis* individuals and grouped them by locality (Fig. 1).

Moreover, the combined analysis of variance revealed that sc_1 and d_2 showed greater variability proving to be useful for species discrimination by location and/or host plant, thus corroborating those setae length could be more discriminating for *O. punicae* and *O. biharensis* characterization (Fig. 4). Despite of the value of setae length for characterization in Tetranychidae species, little information is available. As mentioned by Sandoval (2005), *O. peruvianus* females collected from avocado and cassava trees were discriminated by Principal Components Analysis and differences in v_2, sc_1, c_1, c_2, c_3, d_1, d_2, e_1, e_2, f_1, f_2 and h_3 were observed in *O. peruvianus* populations from both host plant species collected from same location. Similar to our results, setae analysis showed to be a valuable basis for *Oligonychus perseae* female characterization, a species commonly found in avocado trees and often misidentified as *O. peruvianus*.

Other idiosomal morphological characters have been used also for characterization of Tetranychidae species. According to Boudreaux and Dosse (1963), cuticle lobe shape can be used for species characterization. Hence, Hance *et al.* (1998) obtained valuable information to differentiate races of *Tetranychus urticae* Koch and *Tetranychus cinnabarinus* (Boisduval).

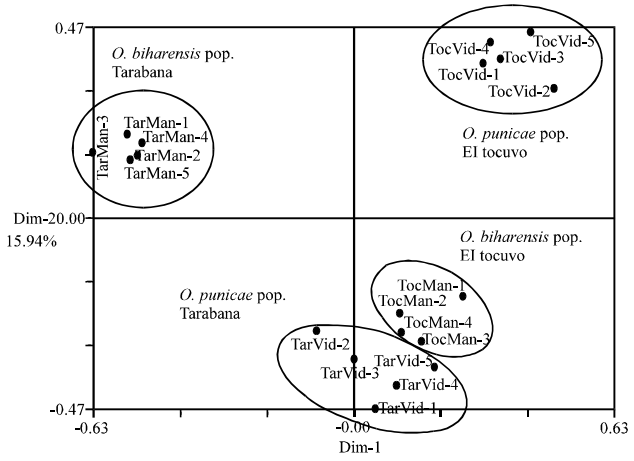

Fig. 4: Principal Coordinate Analysis (PCA) graphic based on RAPD data from *O. punicae* (VID) and *O. biharensis* (MAN) from localities Tarabana (TAR) and El Tocuyo (TOC)

RAPD analysis: Based on present results, primer OPB10 showed to be more liable to amplify DNA polymorphism sectors in *O. punicae* and *O. biharensis* populations from TAR and TOC, thus this primer could be used for discriminating these two *Oligonychus* species. Primer OPB13 showed more restricted potential for species-specific discrimination. Similarly, Osakabe *et al.* (2000) found that out of 40 primers used to evaluate genetic variability in *Panonychus citri* (McGregor), only OPA01, OPB10 and OPB12 yielded diagnostic bands, however, clonation was exclusively achieved in polymorphic bands by using primer OPB10. Garg *et al.* (2009) showed usefulness of RAPD technique to elucidate the complementariness approaches to make diversity analysis more explanatory and powerful for optimum genetic amelioration and effective conservation of genotypic variability.

Although initiator OPB10 yielded the higher polymorphism in *O. punicae* and *O. biharensis* populations; we recommend more detailed studies of these two species including a greater number of individuals, locations and/or host plants to improve the technique.

Although previous studies have demonstrated usefulness of primer OPB10 as molecular markers detector in mites, we recommend the use of more specific primers to detect genetic differences between *O. punicae* and *O. biharensis*. Furthermore, due to RAPD-PCR technique measures DNA polymorphism in a random pattern; value of each band may not have the same weight in an evolutionary context (Hance *et al.*, 1998) as in other more advanced molecular techniques which consuming more time and resources (Ndjiondjop *et al.*, 2006). Thus, variability obtained by the RAPD technique is considered a preliminary result for the two populations of *Oligonychus* studied.

Genetic variation in *O. punicae* and *O. biharensis*: Although the dendrogram constructed for all individuals using cluster analysis (UPGMA) was able to separate mite populations per location, it showed no separation by species. *O. punicae* and *O. biharensis* populations from locality TAR showed higher similarity between them than with their respective homologous population. Accordingly, Paulauskas *et al.* (2006) found that *Ixodes ricinus* L. populations from different localities in Norway and Lithuania were not clearly separated in the dendrogram constructed from genetic distances based on RAPD.

Lower variability observed in *O. biharensis* collected in Tarabana (TAR) could be related to limitative gen flux between individuals due to low population levels were observed in this population. Previous studies have demonstrated that genetic differentiation in *T. urticae* populations laid on density and geographical distance between populations but not on colonized host plant species (Tsagkarakou *et al*., 1998, 1999; Hinomoto and Takafuji, 1995). On the other hand, Tsagkarakou *et al*. (1999) found that genetic differentiation was positive and significantly correlated with geographic distance between populations in field and greenhouses and genetic exchange increased as population density get higher in a microgeographical habitat (<100 m^2).

CONCLUSION

Present results confirm usefulness of morphological characters for species characterization purposes, mainly in case of morphologically similar Tetranychid species, however molecular studies to determine genetic variations prove to be an auxiliary tool to determine molecular variations in tetranychid species of economic importance. It is therefore recommended that more detailed studies involving other molecular techniques and more populations to better understand variability of these mite populations.

ACKNOWLEDGMENTS

To Instituto de la Uva (IUVA) and Estación Experimental Miguel Luna Lugo (Universidad Centroccidental Lisandro Alvarado) for helping to this research.

REFERENCES

Bolland, H.R., J. Gutierrez and C.H.W. Fletchmann, 1998. World Catalogue of the Spider Mite Family (Acari: Tetranychidae). Brill, Leiden, Boston, Koln, pp: 392.

Boudreaux, H.B. and G. Dosse, 1963. The usefulness of new taxonomic characters in females of the genus *Tetranychus* Dufour. Acarologia, 5: 13-33.

Carbonelle, S., T. Hance, A. Migeon, P. Baret, S. Cros-Arteil and N. Navajas, 2007. Microsatellite markers reveal spatial genetic structure of *Tetranychus urticae* (Acari: Tetranychidae) populations along a latitudinal gradient in Europe. Exp. Appl. Acarol., 41: 225-241.

Cenis, J.L., 1993. Identification of the four major *Meloidogyne* spp. by random amplified polymorphic DNA (RAPD-PCR). Phytopathology, 83: 76-80.

Cerna, E., M.H. Badii, Y. Ochoa, L.A. Aguirre and J. Landeros, 2009. Life tables of Oligonychus punicae Hirst (Acari: Tetranychidae) in avocado leaves in the hass, fuerte and criollo cultivars. Universidad y Ciencias, 25: 133-140.

Chen, W., Y. Fu, F. Zhang and Z. Peng, 2005. Effect of different varieties of litchi on the development and reproduction of *Oligonychus biharensis* (Hirst). Syst. Appl. Acarol., 10: 11-16.

Cumagun, C.J.R., H.P. Parzies and T. Miedaner, 2007. Genetic variation and segregation of DNA polymorphisms in gibberella zeae detected with AFLP and RAPD markers. Asian J. Plant Sci., 6: 1174-1181.

Garg, R.K., P. Sairkar, N. Silawat, N. Vijay, N. Batav and N.N. Mehrotra, 2009. Genetic diversity between two populations of *Heteropneustes fossilis* (Bloch) using RAPD profile. Int. J. Zool. Res., 5: 171-177.

Gettinger, D. and R.D. Owen, 2000. *Androlaelaps rotundus* Fonseca (Acari: Laelapidae) associated with akodontine rodents in paraguay: A morphometric examination of a pleioxenous ectoparasite. Ver. Bras. Biol., 60: 425-434.

Gould, W.P. and A. Raga, 2002. Pest in Guava. In: Tropical Fruits Pest and Pollinators: Biology, Economic Importance, Natural Enemies and Control, Pena, J.E., J.L. Sharp and M. Wysoki (Eds.). CAB International, Wallingford, ISBN: 0-85199-344-2, pp: 295-314.

Hance, T., P. Neuberg and C. Noel-Lastelle, 1998. The use of fecundity, lobe biometry and the RAPD-PCR technique in order to compare strains of *Tetranychus* sp. Exp. Applied Acarol., 22: 649-666.

Helle, W. and W. Overmeer, 1985. Rearing Techniques. In: Spider Mites: Their Biology, Natural Enemies and Control, Helle, W. and M.W. Sabelis (Eds.). Elsevier Science Publishers, B.V. Amsterdam, ISBN: 0-444-42372-9, pp: 331-335.

Hinomoto, N. and A. Takafuji, 1995. Genetic changes in the population structure of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), on vinyl-house strawberry. Applied Entomol. Zool., 30: 521-528.

Hlaoua, W., N. Horrigue-Raouani, D. Fouville and D. Mugniery, 2008. Morphological and molecular characterisation of potato cyst nematode populations from Tunisia and survey of their probable geographical origin. Biotechnology, 7: 651-659.

Jeppson, L.R., H.H. Keifer and E.W. Baker, 1975. Mites Injurious to Economic Plants. University of California Press, Berkeley, Los Angeles, pp: 614.

Krantz, G.W., 1978. A Manual of Acarology. 2nd Edn., Oregon State University Book Stores, Inc., USA., pp: 509.

Lakshmi, P., P.A.A. Khan, P.N. Reddy, K. Lakshminarayana and S. Ganapaty, 2008. Genetic relationship among *Tephrosia* species as revealed by RAPD analysis. Asian J. Biol. Sci., 1: 1-10.

Magalhaes, S., M.R. Forbes, A. Skoracka, M. Osakabe, C. Chevillon and K.D. Mccoy, 2007. Host race formation in the Acari. Exp. Appl. Acarol., 42: 225-238.

Meyers, L.A. and J.J. Bull., 2002. Fighting change with change: Adaptive variation in an uncertain world. Trends Ecol. Evol., 17: 551-557.

Moder, K., B.C. Schlick-Steiner, F.M. Steiner, S. Cremer, E. Christian and B. Seifert, 2007. Optimal species distinction by discriminant analysis: Comparing established methods of character selection with a combination procedure using ant morphometrics as a case study. J. Zool. Syst. Evol. Res., 45: 82-87.

Navajas, M., J. Gutierrez, O. Bonato, H.R. Bolland and S. Mapangou-Divassa, 1994. Intraespecific diversity of the cassava green mite *Mononychellus progresivus* (Acari: Tetranychidae) using comparisons of mitochondrial and nuclear ribosomal DNA sequences and cross-breeding. Exp. Applie Acarol., 18: 351-360.

Ndjiondjop, M.N., K. Semagn, M. Cissoko, H. Tsunematsu and M. Jones, 2006. Genetic relationships among rice varieties based on expressed sequence tags and microsatellite markers. Asian J. Plant Sci., 5: 429-437.

Ochoa, R., H. Aguilar and C. Vargas, 1994. Phytophagous Mites of Central America: An illustrated guide. CATIE, Turrialba, pp: 234.

Osakabe, M., N. Hinomoto, S. Toda, S. Komazaki and K. Goka, 2000. Molecular cloning and characterization of a microsatellite locus found in an RAPD marker of a spider mite, *Panonychus citri* (Acari: Tetranychidae). Exp. Appl. Acarol., 24: 385-395.

Patra, A.P., A.K. Mukherjee and L. Acharya, 2011. Comparative study of RAPD and ISSR markers to assess the genetic diversity of betel vine (*Piper betle* L.) in Orissa, India. Am. J. Biochem. Mol. Biol., 1: 200-211.

Paulauskas, A., J. Radzojevskaja, O. Rosef, J. Turcinaviciene, D. Ambrasiene and D. Marareviciute, 2006. Genetic variation of ticks (*Ixodes ricinus* L.) in the Lithuanian and Norwegian populations. Exp. Appl. Acarol., 40: 259-270.

Pritchard, A.E. and E.W. Baker, 1955. A revision of the spider mite: Family tetranychidae. Memoirs Ser., 2: 472-472.

Rodrigues, J.C., M. Gallo-Meagher, R. Ochoa, C.C. Childers and B.J. Adams, 2004. Mitochondrial DNA and RAPD polymorphisms in the haploid mite *Brevipalpus phoenicis* (Acari: Tenuipalpidae). Exp. Appl. Acarol., 34: 275-290.

Rohlf, F.J., 1992. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Version 1.70, Exeter Software, Setauket, New York.

SASJMP, 2003. The Statistical Discovery Software. SAS Institute Inc., Cary, NC., USA.

Sandoval, M.F., 2005. Ecological and taxonomical aspects of *Oligonychus peruvianus* (McGregor, 1917) (Acari: Tetranychidae) on avocado (*Persea americana* Mill.), Aragua. Magister Scientiarum Dissertation, Facultad de Agronomia. Universidad Central de Venezuela.

Sneath, P.H.A. and R.R. Sokal, 1973. Numerical Taxonomy: The Principles and Practice of Numerical Classification. W.H. Freeman and Co., San Francisco.

Tsagkarakou, A., M. Navajas, J. Lagnel and N. Pasteur, 1997. Population structure in the spider mite *Tetranychus urticae* (Acari: Tetranychidae) from crete based on multiple allozymes. Heredity, 78: 84-92.

Tsagkarakou, A., M. Navajas, P. Papaioannou-Souliotis and N. Pasteur, 1998. Gene flow among *Tetranychus urticae* (Acari: Tetranychidae) populations in Greece. Mol. Ecol., 7: 71-79.

Tsagkarakou, A., M. Navajas, F. Rousset and N. Pasteur, 1999. Genetic differentiation in *Tetranychus urticae* (Acari: Tetranychidae) from greenhouses in France. Exp. Applied Acarol., 23: 365-378.

Vasquez, C., O. Aponte, J. Morales, M.E. Sanabria and G. Garcia, 2008. Biological studies of *Oligonychus punicae* (Acari: Tetranychidae) on grapevine cultivars. Exp. Appl. Acarol., 45: 59-69.

Vasquez, C., A. Mondragon, M. Davila and O. Aponte, 2009. Phytophagous mites (Tetranychoidea: Tetranychidae, Tenuipalpidae) from natural vegetations in Lara. Venezuela. Biota Neotropica, 9: 55-58.

Yli-Mattila, T., S. Paavanen-Huhtala, B. Fenton and T. Tuovinen, 2000. Species and strain identification of the predatory mite *Euseius finlandicus* by RAPD-PCR and ITS sequences. Exp. Appl. Acarol., 24: 863-880.

Zhang, Z.Q., 2003. Mites of Greenhouses: Identification, Biology and Control. CABI, New York, pp: 240.