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Genetic variability of *Dactylopius opuntiae* (Hemiptera, Dactylopiidae) on forage cactus in northeast Brazil

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ABSTRACT. The carmine cochineal *Dactylopius opuntiae* is a key pest in productive fields of forage cactus in Pernambuco, Brazil. Species identification by means of molecular markers assists in understanding the genetic profile, underpins morphological characterization, and supports the monitoring of populations in integrated management programs designed to control this pest. We evaluated the genetic variability of natural populations of *D. opuntiae*. Genetic variability was analyzed with ISSR and RAPD primers in 24 populations from 12 municipalities of Pernambuco State in Brazil. Morphological characterization confirmed that *D. opuntiae* was the only cochineal species present in all samples. Nine ISSR primers and six RAPD produced a total of 62 and 58 polymorphic fragments, respectively. Both types of markers showed an average genetic similarity of 80% regardless of the geographic origin of samples. The low genetic variability demonstrates a high degree of relatedness among these *D. opuntiae* populations.

Key words: Dactylopiidae; Genetic markers; *Opuntia ficus indica*; Polymorphism

INTRODUCTION

Northeast Brazil is the largest storehouse of cactus in the world, with approximately 600,000 hectares represented by the species *Opuntia ficus indica* (L.) Mill and *Nopalea cochenillifera* Salm-Dyck (Silva et al., 2010; Nunes, 2011). The wild cochineal *Dactylopius opuntiae* Cockerell (Hemiptera; Dactylopiidae) is considered to be a key pest in crops of significant economic importance (Lacerda et al., 2011). This insect primarily affects the cultivars of the genus *Opuntia* (Flores-Flores and Tekelenburg, 2001) and its biotic potential and invasive growth has caused the destruction of approximately 100,000 hectares in Pernambuco and Paraíba and, to a lesser extent, in Rio Grande do Norte and Ceará (Lopes et al., 2009).

Dactylopius comprises the single genus in the family Dactylopiidae, forming a homologous group of phytophagous Hemiptera that comprises ten species (Pérez-Guerra and Kosztarab, 1992; Portillo and Vigueras, 2006). *D. coccus* Costa is a species of great socioeconomic importance, whose adult females serve as feedstock for storing 19-24% (dry weight) of carminic acid, a high-quality dye used worldwide in many industrial sectors (Aldama-Aguilera et al., 2005). In Brazil, the occurrence of *Dactylopius* species is still widely debated, especially in the Northeast. Reports dating from 1968 highlight the occurrences of *D. ceylonicus*, *D. indicus*, and *D. subterraneus* in semiarid regions of Pernambuco (Silva et al., 1968 apud Warumby et al., 2005). However, redescriptive studies on the biosystematics of the Dactylopiidae presented by Pérez-Guerra (1991), Perez-Guerra and Kosztarab (1992), and Ben-Dov (2006) prove the existence of *D. opuntiae* in the city of Arcoverde, Pernambuco, since 1973. These authors also revealed the synonymy between the two former species, while *D. subterraneus* was confused with *Planococcus ficus* Signoret (Hemiptera: Pseudococcidae) (Walton and Pringle, 2004).

The taxonomic identifications of the Dactylopiidae contribute to the morphological characteristics of adult females mainly by the number, distribution, and types of pores and setae (Miller and Kosztarab, 1979; Pérez-Guerra, 1991). However, the complexity of interactions between phytophagous insects and their hosts, requirements of the insect's adult stage, and environmental factors are parameters that limit the accuracy of morphological analysis (Miller and Kosztarab, 1979). Therefore, molecular markers such as random amplified polymorphic DNA-PCR (RAPD-PCR) and inter-simple sequence repeat-PCR (ISSR-PCR) are powerful tools in studies on the genetic variability of insect species (Lima et al., 2002; Souza et al., 2008, Silva et al., 2009; Auad et al., 2010; Helmi and Khafaga, 2011). Molecular characterization, in turn, allows evaluations of the polymorphism within and between *Dactylopius* species based on the direct analysis of the DNA molecule, permitting access to a wide genome region. The RAPD and ISSR techniques have been widely used for genotyping purposes. Their detected fragments tend to be monomorphic when the genetic variability between organisms is reduced. Conversely, the variability of the fragments increases when high levels of polymorphisms are detected (Iruela et al., 2002; Martins et al., 2003). Another advantage of using these techniques is that the molecular descriptors are usually corroborated with morphological data (Wang et al., 1998; Scarano et al., 2002).

So far, there are few molecular studies available in the literature on *D. opuntiae* that can highlight some other related species of the genus *Dactylopius* (von Dohlen and Moran, 1995; Rodriguez et al., 2001; Cook et al., 2002; Ramírez-Puebla et al., 2010). However, only the studies of García et al. (1999, 2000) used RAPD markers to detect polymorphisms in some wild species and *D. coccus*. This is the first study using RAPD and ISSR markers for the species *D. opuntiae*, whose information has not yet been reported in the literature. Thus, the objective of this study was to evaluate the genetic variability of *D. opuntiae* populations using RAPD and ISSR markers in productive areas of forage cactus in the microregions of “Agreste” and “Sertão” of Pernambuco, Brazil.

MATERIAL AND METHODS

This study was conducted in Laboratórios de Entomologia e de Genoma, Instituto Agronômico de Pernambuco (IPA), Recife, Pernambuco. A total of 24 accessions of *D. opuntiae* from forage cactus infested by the pest from 12 municipalities in the microregions located in Zone 24, Central Meridian 39, Pernambuco, Brazil, were used in this study (Figure 1, Table 1).

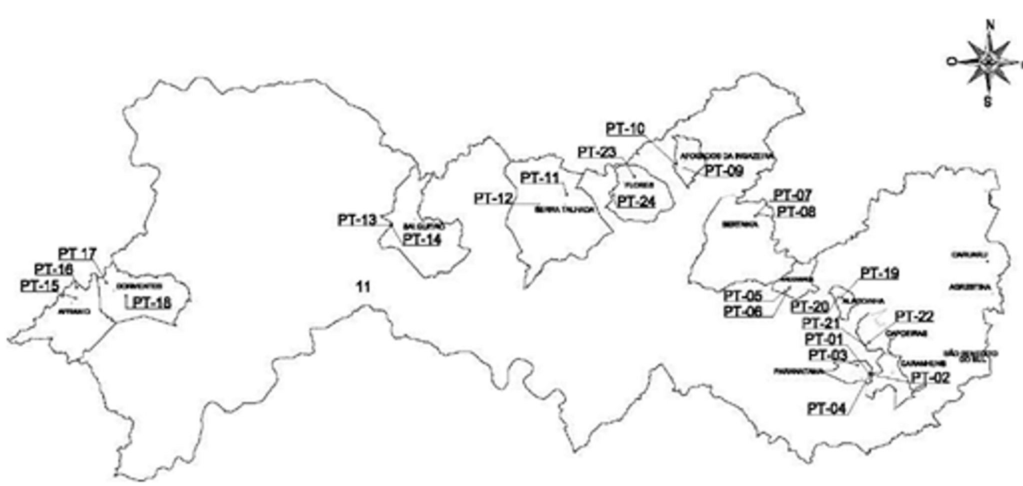


Figure 1. Map of Pernambuco. Zone 24, Central Meridian 39 representing the locations of the 24 accessions of *Dactylopius opuntiae* on *Opuntia ficus indica* in the “Agreste” and “Sertão” microregions of Pernambuco, Brazil, from 2009 to 2010.

Table 1. Locations of the 24samples *Dactylopius opuntiae* in Pernambuco.

Microregions	Municipalities	Samples	Properties	Latitude - Longitude
Garanhuns	Garanhuns	1	(GAR 1) Sítio Mocós	08°53'.310" S - 36°37'.062" W
		2	(GAR 2) Sítio Mimozinho	08°53'.722" S - 36°35'.680" W
	Paranatama	3	(PARAN 1) Sítio Nambi	08°50'.745" S - 36°40'.675" W
		4	(PARAN 2) Sítio Rego	08°54'.013" S - 36°37'.013" W
Sertão do Moxotó	Arcoverde	5	(SM) Sítio Salobro	08°26'.978" S - 37°04'.855" W
		6	(SM) EEA - IPA*	08°26'.064" S - 37°03'.390" W
	Sertânia	7	(SM) Sítio Queimada	08°02'.473" S - 37°14'.851" W
		8	(SM) Sítio Rinaldo	08°93'.703" S - 37°11'.812" W
Sertão do Pajeú	Afogados da Ingazeira	9	(SP) Poço Moleque	07°92'.819" S - 37°35'.428" W
		10	(SP) Santo Antônio	07°45'.885" S - 37°41'.030" W
	Serra Talhada	11	(SP) Fazenda Saco	07°55'.74.2" S - 38°17'13.3" W
		12	(SP) Fazenda Cedro	07°58'.550" S - 38°26'.040" W
Salgueiro	Salgueiro	13	(SAL) Sítio Paraguaçu	08°30'.961" S - 34°33'.666" W
		14	(SAL) Sítio Caixito	08°05'.766" S - 39°14'.486" W
Petrolina	Afrânio	15	(PET) A-I (Sítio Mucambo)	08°29'.203" S - 40°58'.486" W
		16	(PET) A-II (Pedrinha)	08°25'.949" S - 40°58'.316" W
	Dormentes	17	(PET) D-I (Bargado)	08°24'.197" S - 40°48'.435" W
		18	(PET) D-II (Sítio José Neto)	08°28'.320" S - 40°42'.168" W
Vale do Ipojuca	Alagoinha	19	(VI) Sítio Riacho	08°29'.056" S - 36°46'.243" W
		20	(VI) Sítio João Henrique	08°28'.665" S - 36°47'.348" W
	Capoeiras	21	(VI) Sítio Mocambo	08°45'.162" S - 36°38'.387" W
		22	(VI) João Carneiro Sobrinho	08°43'.346" S - 36°37'.164" W
Sertão do Pajeú	Flores	23	(SP) Sítio Cajá	07°50'.083" S - 37°54'.459" W
		24	(SP) Sítio Barreiros	07°55'.984" S - 38°01'.380" W

*EEA = Estação Experimental de Arcoverde.

The colonies to obtain adult females were reared on infested cladodes in individual wooden cages (85 x 60 x 50 cm), screened with voile fabric and maintained at 25° ± 0.5°C, 70 ± 10% relative humidity, and a 12-h photoperiod. Females in the pre-oviposition phase from 24 localities were selected and forwarded to the Center for Morphological Identification by Fundação Estadual de Pesquisa Agropecuária (FEPAGRO), based on the description of Pérez-Guerra and Kosztarab (1992).

Extraction and DNA quantification

To remove wax, the adult female specimens were treated with absolute ethanol (99.5%). Approximately 40 mg females were transferred to microvials, after which 600 µL extraction buffer (Nuclei Lysis Solution) was added, followed by immersion in liquid nitrogen to facilitate grinding. DNA was extracted using the Genomic DNA Purification kit (QIAGEN), and DNA concentration was estimated by electrophoresis on a 0.8% (w/v) agarose gel run with 0.5X Tris-borate-EDTA (TBE) and compared with pre-determined amounts of a marker of known molecular weight (Lambda phage DNA; Invitrogen Life Technologies). Gels were stained with Sybr Gold and visualized under ultraviolet light with the aid of a photo documentation system (L.PIX).

DNA amplification with ISSR and RAPD primers

The DNA samples were subjected to PCR with the primers listed in Table 2. A preliminary selection of ISSR primers (University of British Columbia) and RAPD primers (Operon Technologies) was performed. The amplification reactions were performed with GoTaq Colorless Master Mix (2X; Promega) at a 10-μL final volume under the following conditions: DNA polymerase buffer, pH 8.5, 400 μM dNTP, 3 mM $MgCl_2$, 5 μM of each primer, and 10 ng DNA template. The amplification cycle was performed in an MJ Research PCT-100 thermocycler (Perkin Elmer) with the following program: initial denaturation at 95°C for 15 min, followed by 34 cycles at 94°C for 30 s, annealing at 50°C for 45 s and an extension at 72°C for 2 min, and a final extension at 72°C for 7 min. The amplified DNA fragments were separated by electrophoresis on a 1.5% agarose gel in 0.5X TBE buffer, using a 1-kb DNA ladder (Invitrogen) molecular weight marker. Thereafter, the gels were stained with Sybr Gold and visualized under ultraviolet light with the aid of a photodocumentation system (L.PIX).

Table 2. List of RAPD and ISSR primers used in the analysis of genetic variability in populations of *Dactylopius opuntiae*.

	Sequence (5'→3')	Size of fragment (bp)	Total No. / fragment	No. polymorphic bands	No. monomorphic bands	Percentage of polymorphism (%)
Primer (ISSR)						
UBC 01	$(AC)_8T$	500-1700	8	6	2	75.0
UBC 02	$(AG)_8T$	400-1650	4	2	2	50.0
UBC 808	$(AG)_8C$	400-1650	7	5	2	71.4
UBC 810	$(GA)_8T$	400-1900	8	4	4	50.0
UBC 812	$(GA)_8A$	200-1000	3	2	1	66.6
UBC 827	$(AC)_8G$	300-2000	6	4	2	66.6
UBC 836	$(AG)_8YA$	300-1600	10	8	2	80.0
UBC 885	$TCT(GA)_7$	200-1400	8	7	1	87.5
UBC 880	$(GGAGA)_3$	300-1800	8	6	2	75.0
Total = 9			62	44	18	70.9
Primer (RAPD)						
OPA 03	AGTCAG CCA C	300-2100	12	9	3	66.6
OPA 04	AAT CGG GCT G	400-2800	13	11	2	84.6
OPA 10	GTG ATC GCA G	300-2600	8	6	2	75.0
OPA 20	GTT GCG ATC C	300-2000	10	7	3	70.0
OPB 01	GTT TCG CTC C	400-2900	7	5	2	71.4
OPB 07	GGT GAC GCA G	400-3000	8	6	2	75.0
Total = 6			58	44	14	75.8

Statistical analysis

The data obtained from amplifications with ISSR and RAPD markers were analyzed by the Numerical Taxonomy System of Multivariate Programs (NTSYS-pc) (Bussab et al., 1990; Rohlf, 2000) and introduced in the form of binary variables, namely 1 for the presence of a band and 0 for the absence. Thus, the program was used to build a similarity matrix based on the Jaccard coefficient. The constructed similarity matrix was used to generate a dendrogram by the unweight pair group method with arithmetical average (UPGMA) clustering method.

RESULTS

Fifteen primers were selected from among the 30 proposed, 9 ISSR and 6 RAPD, as they showed the best polymorphic patterns. The 15 ISSR primers and other RAPD primers were monomorphic and were thus excluded from the analyses. The ISSR primer UBC 885 (Figure 2) stood out among the rest by generating the highest percentage of polymorphism (87.5%), thus indicating distinct patterns within *D. opuntiae* species. A total of 62 ISSR fragments were produced, and the number of fragments per primer was 7, ranging between 4 and 10. The size of these fragments ranged from 200 to 2000 bp (Table 2).

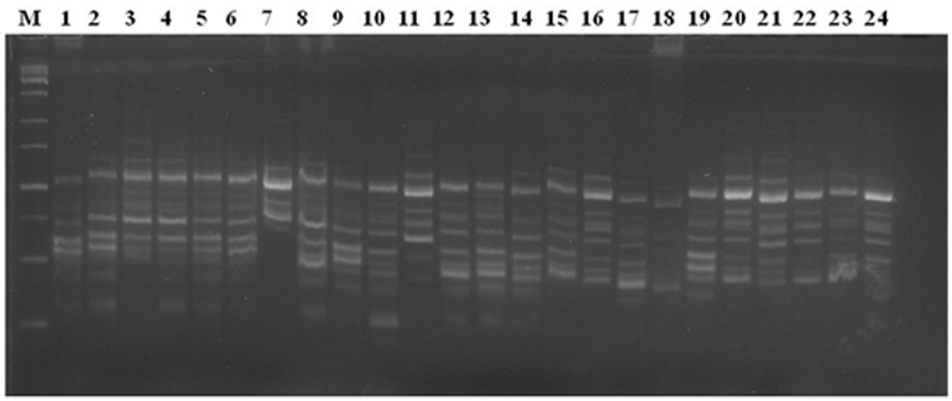


Figure 2. Amplification profiles of the ISSR regions with the primer UBC 885. *Lane M* = 1-kb molecular weight marker. *Lanes 1* to *24* = DNAs of *Dactylopius opuntiae* populations situated in Table 1.

Among the six RAPD primers selected, OPA 04 showed the highest percentage of polymorphism (84.6%) (Figure 3). A total of 58 fragments were obtained, and the number of fragments per primer ranged from 7 (OPB 01) and 13 (OPA 04), with a mean of 9.6. The fragment sizes ranged from 300 to 3000 bp (Table 2).

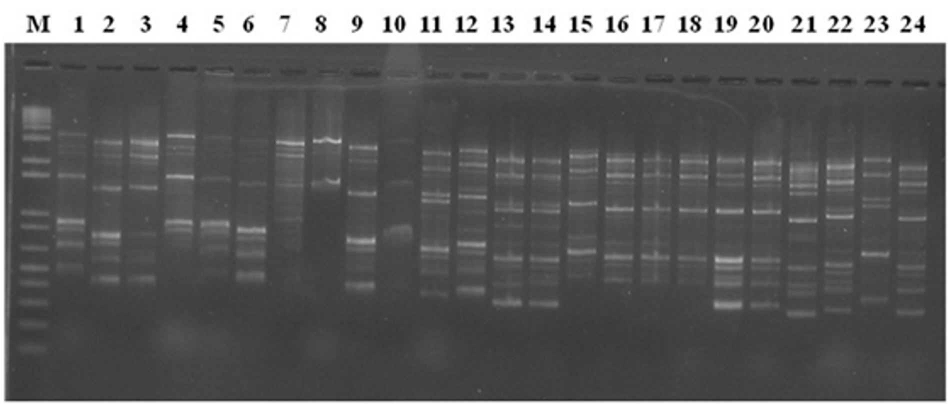
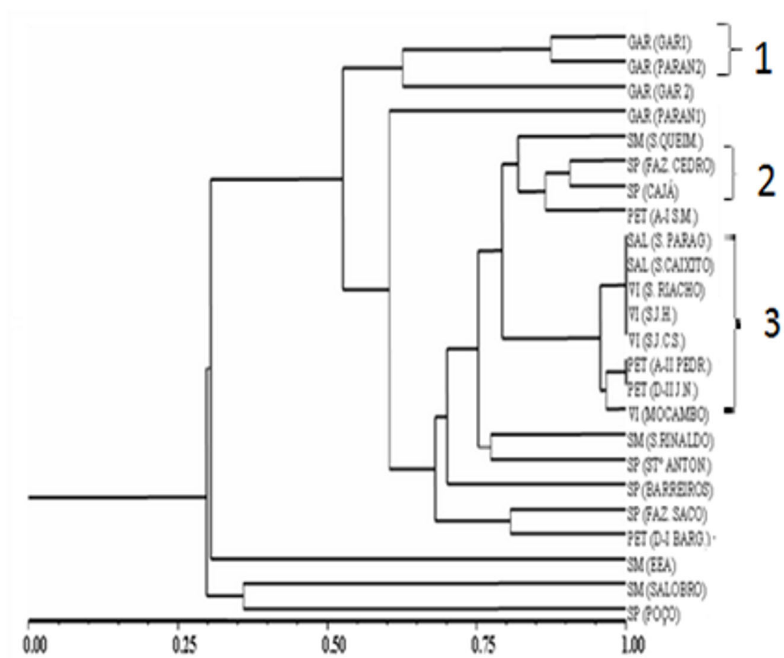


Figure 3. RAPD amplification profiles with the primer OPA 04. *Lane M* = 1-kb molecular weight marker. *Lanes 1* to *24* = DNAs of the populations located in Table 1.

The dendrogram obtained by UPGMA (Figure 4) of the four RAPD markers showed the formation of three clusters at 80% similarity level of fragment size. The first group consisted of two accessions of *D. opuntiae* from the municipalities of Garanhuns (GAR 1) and Paranatama (PARAN 2), which showed 87% similarity to each other, while Garanhuns (GAR

2) showed 61% similarity to the other two. The second group consisted of *D. opuntiae* populations from "Sertão" do Pajeú (SP, Fazendas Cedro and Cajá). These populations showed 90% similarity to each other; however, when compared with those in "Sertão" do Moxotó (SM, Sítio Queimada), they showed 81% similarity and for Petrolina [PET, A-I (Sítio Mucambo)], 86% similarity. The third group consisted of cochineals from Salgueiro (SAL, Sítios Paraguaçu and Caixito), Vale do Ipojuca (VI, Sítios Riacho, Sítio João Henrique and João Carneiro Sobrinho) and Petrolina [PET A-II (Pedrinha)]. The third group showed the highest similarity (100 %) and was considered to be homologous.



Jaccard's coefficient

Figure 4. Dendrogram obtained by the UPGMA method based on the genetic distances by RAPD-PCR marker, showing the existing relationship among *Dactylopius* populations on forage cactus in the municipalities of "Agreste" and "Sertão" in Pernambuco. The location codes are listed in Table 1.

The UPGMA dendrogram determined by ISSR-PCR showed the formation of four groups, 80% at the fragment size similarity level (Figure 5). The first group comprised cochineals grouped into Garanhuns (GAR 1, GAR 2) and "Sertão" do Moxotó (SM, Salobro), and showed 90% similarity to each other, forming the maximum similarity obtained by the Jaccard coefficient and those of "Sertão" do Moxotó (EEA) with less than 75% similarity. Populations of Garanhuns (GAR 1, GAR 2) showed 66% similarity compared to other representatives of this group.

In the second cluster, samples of Petrolina [PET, AI (SM)] and "Sertão" do Pajeú (SP, Barreiros) showed 82% similarity, while the "Sertão" do Pajeú (SP, Cajá) and Garanhuns

(GAR, PARAN 1) showed lower similarity: 80 and 74%, respectively, compared to the other two. The cochineals of Salgueiro (SAL, Sítios Paraguaçu and Caixito) showed 74% similarity between each other. It was also found that the cochineals of "Sertão" do Pajeú (SP, Santo Antônio and Fazenda Cedro) had 76% similarity between their fragment sizes.

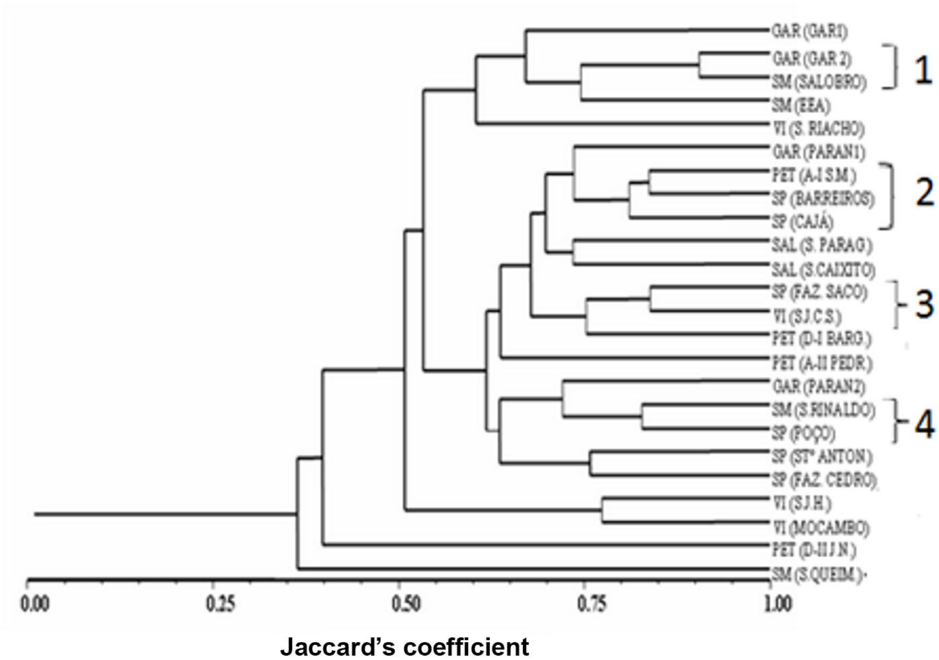


Figure 5. Dendrogram obtained by UPGMA method based on the genetic distances using ISSR-PCR marker showing the relationship among *Dactylopius* populations in forage cactus in the municipalities of "Agreste" and "Sertão" of Pernambuco. The location codes are listed in Table 1.

The third group formed by the *D. opuntiae* populations of "Sertão" of Pajeú (SP, Fazenda Saco) and Vale do Ipojuca (Sítio João Carneiro Sabrinho), showed 84% similarity between each other, while those of Petrolina [PET, D-I (Bargado)] showed 75% similarity to the other two. The fourth group was formed by accessions of "Sertão" do Moxotó (SM, Sítio Rinaldo) and "Sertão" do Pajeú (SP, Poço), showing 83% similarity. Samples of Petrolina (PET, D-II JN, Sítio José Neto), "Sertão" do Moxotó (SM, Sítio Queimada), Petrolina [PET, A-II (Pedrinha)], and Vale do Ipojuca (VI, Sítio Riacho) did not form groups at 80% similarity regarding fragment size, indicating genetic differentiation compared to other accessions.

DISCUSSION

The 24 populations of carmine cochineals were morphologically characterized and deposited in the collection of the Museum Ramiro Costa Gomes (MRGC; FEPAGRO, RS). The results confirm the presence of *D. opuntia* in all areas of *O. ficus indica* evaluated in Pernambuco.

The results show that the primers used for RAPD and ISSR analysis of the cochineal carmine *D. opuntiae* were satisfactory in detecting polymorphisms. Similar results were obtained by Miranda et al. (2012), who studied 104 colonies of bees endemic to "Caatinga", using 10 ISSR primers and obtained a total of 109 bands and an average of 72.47% polymorphism. However, genetic variability showed a moderate population structure. Similarly, Radjabi et al. (2012) studied various races of the silkworm *Bombyx mori* Linn. (Lepidoptera: Bombycidae) and obtained from five selected ISSR primers 81 polymorphic fragments and 77.77% polymorphism. Souza et al. (2008) assessed the genetic diversity of populations *Zabrotes subfasciatus* Boh. (Coleoptera, Bruchidae) and found 52 polymorphic fragments and an average polymorphism of 83.82% but low levels of genetic differentiation. In contrast, Borba et al. (2005), studying strains of *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae), detected a high percentage (96%) of polymorphism. Among the insects widely investigated by molecular techniques, the silkworm *B. mori* is a prominent model system and recognized in several countries, which contributes to the improvement of breeds of this insect (Bakkappa et al., 2011).

Studies and polymorphisms related to the potential spread of *Dactylopius* species were carried out by García et al. (1999, 2000) when RAPD primers were used for the first time. We evaluated populations of nymphs I and adults of *D. coccus* and *Dactylopius* spp wild being obtained fragments of different sizes. Most of these patterns were monomorphic, with a discrete number of fragments showing polymorphism.

Bemisia tabaci (Genn.) (Hemiptera: Aleyrodidae) was studied by Fontes et al. (2010), who obtained a total of 71 loci, an average of 4.6 polymorphic bands and 80.6% polymorphism. Silva et al. (2009) reported high polymorphism (89.2%) of *B. tabaci* populations in okra, beans, and chili, as well as high genetic similarity (80.76 and 44%) tested with 12 RAPD primers. Rampelotti et al. (2008) detected in populations of the bug-of-thatched rice, *Tibraca limbativentris* Stal (Hemiptera: Pentatomidae), a high level of polymorphism (98%) with the primer OPA 04, considered the most polymorphic.

This study showed that RAPD markers, compared to ISSR, were better at grouping the populations of *D. opuntiae*, forming groups with 100% genetic similarity. However, neither marker discriminated genetically the 24 populations studied in different geographical regions of Pernambuco. Thus, the low genetic divergence detected points to the discrete genetic differences between populations of *D. opuntiae*, suggesting that other PCR methods need to be investigated to obtain new answers regarding their dispersal, adaptation, and management control.

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REFERENCES

Aldama-Aguilera C, Llanderal-Cázares C, Soto-Hernández M and Castillo-Márquez LE (2005). Producción de grana-cochinilla (*Dactylopius coccus* Costa) en plantas de nopal a la intempérie y en microtúneles. *Agrociencia* 39: 161-171.

Auad AM, Domingues R, Machado MA, Souza LS, et al. (2010). Genetic variability of *Mahanarva* sp (Hemiptera: Cercopidae) collected from different sites in Brazil. *Genet. Mol. Res* 9: 1005-1010.

Bakkappa S, Talebi E and Subramanya G (2011). Role of molecular markers (RAPD & ISSR) in silkworm conservation. *Int. J. Adv. Biol. Res.* 1: 1-7.

Ben-Dov Y (2006). A Systematic Catalogue of Eight Scale Insect Families (Hemiptera: Coccoidea) of the World. Elsevier, Amsterdam.

Borba RS, Garcia MS, Kovaleski A, Oliveira AC, et al. (2005). Dissimilaridade genética de linhagens de *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) através de marcadores moleculares ISSR. *Neotrop. Entomol.* 34: 565-569.

Bussab WO, Miazaki ES and Andrade DF (1990). Introdução à Análise de Agrupamentos. Associação Brasileira de Estatística, São Paulo.

Cook LG, Gullan PJ and Trueman HE (2002). A preliminary phylogeny of the scale insects (Hemiptera: Sternorrhyncha: Coccoidea) based on nuclear small-subunit ribosomal DNA. *Mol. Phylogenet. Evol.* 25: 43-52.

Flores-Flores V and Tekelenburg A (2001). Produção de Corante Dacti (*Dactylopius coccus*). Agroecologia, Cultivo e Utilizações da Palma Forrageira. SEBRAE, João Pessoa, 169-186.

Fontes FVHM, Colombo CA and Lourenção AL (2010). Caracterização molecular e divergência genética de *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) em diferentes culturas e locais de cultivo. *Neotrop. Entomol.* 39: 221-226.

García GF, Rojas MA, Petriz EE and Hernández HF (1999). *Dactylopius coccus* y *Dactylopius* sp.: detección de polimorfismos en el DNA usando RAPD-PCR y comparación entre especies. *Imaggen* (*número especial dedicado a la investigación*), *Universidad Simón Bolívar* 48: 16-22.

García GF, Rojas MA and Hernández HF (2000). Descripción de marcadores genéticos que permiten identificar poblaciones y migraciones del parásito del nopal *Dactylopius* sp. (cochinilla silvestre). *Biologia* (*número especial dedicado a la investigación*) 2: 15-19.

Helmi A and Khafaga AF (2011). Molecular Fingerprinting of Certain Cereal Aphids in Egypt (Hemiptera: Sternorrhyncha: Aphididae) Using RAPD and ISSRs Markers. *J. Entomol.* 8: 327-340.

Iruela M, Rubio J, Cubero JI, Gil J, et al. (2002). Phylogenetic analysis in the genus *Cicer* and cultivated chickpea using RAPD and ISSR markers. *Theor. Appl. Genet.* 104: 643-651.

Lacerda CA, Santos VF, Borges LR, Santos EA, et al. (2011). Utilização de produtos alternativos para controle da cochonilha-do-carmim *Dactylopius opuntiae* (Cockerell) em palma forrageira. *Pesq. Agropec. Pernamb.* 16: 31-41.

Lima LHC, Campos L, Moretzsohn MC, Navia D, et al. (2002). Genetic diversity of *Bemisia tabaci* (Genn.) Populations in Brazil revealed by RAPD markers. *Genet. Mol. Biol.* 25: 217-223.

Lopes EB, Albuquerque IC, Brito ICH and Batista JL (2009). Velocidade de dispersão de *Dactylopius opuntiae* em palma gigante (*Opuntia ficus-indica*). *Eng. Amb. Pesq. Technol.* 6: 644-649.

Martins M, Tenreiro R and Oliveira MM (2003). Genetic relatedness of Portuguese almond cultivars assessed by RAPD and ISSR markers. *Plant Cell Rep.* 22: 71-78.

Miller DR and Kosztarab M (1979). Recent advances in the study of scale insects. *Annu. Rev. Entomol.* 24: 1-27.

Miranda EA, Batalha-Filho H, Oliveira PS, Alves RMO, et al. (2012). Genetic diversity of *Melipona mandacaia* SMITH 1863 (Hymenoptera, Apidae), an endemic bee species from brazilian caatinga, using ISSR. *Psyche* 2012: 1-6.

Nunes CS (2011). Usos e aplicações da palma forrageira como uma grande fonte de economia para o semiárido nordestino. *Rev. Verde* 6: 58-66.

Pérez-Guerra GM del C (1991). Biosystematics of the Family Dactylopiidae (Homoptera: Coccinea) with Emphasis on the Life Cycle of *Dactylopius coccus* Costa. Doctoral thesis, Virginia Polytechnic Institute and State University, Blacksburg.

Pérez-Guerra G and Kosztarab M (1992). Biosystematics of the family Dactylopiidae (Homoptera: Coccinea) with emphasis on the life cycle of *Dactylopius coccus* Costa (Studies on the morphology and systematics of scale insects). *Bull. Virginia Agr. Exp. Station* 16: 1-90.

Portillo MI and Vigueras AL (2006). A review on the cochineal species in México, host and natural enemies. *Acta Hort.* 728: 249-256.

Radjabi R, Sarafrazi A, Tarang A, Kamali K, et al. (2012). Intraspecific biodiversity of Iranian local races of silkworm *Bombyx mori* by ISSR (inter-simple sequence repeat) molecular marker. *World J. Zool.* 7: 17-22.

Ramírez-Puebla ST, Rosenblueth M, Chávez-Moreno CK, de Lyra MC, et al. (2010). Molecular phylogeny of the genus *Dactylopius* (Hemiptera: Dactylopiidae) and identification of the symbiotic bacteria. *Environ. Entomol.* 39: 1178-1183.

Rampelotti FT, Ferreira A, Tcacenco FA, Martins JFS, et al. (2008). Diversidade genética de *Tibraca limbativentris* Stal. (Hemiptera: Pentatomidae) de Santa Catarina e do Rio Grande do Sul, usando marcadores RAPD. *Neotrop. Entomol.* 37: 20-29.

Rodriguez LC, Méndez MA and Niemeyer HM (2001). Direction of dispersion of cochineal (*Dactylopius coccus* Costa) within the Americas. *Antiquity* 75: 73-77.

Rohlf FJ (2000). NTSYS-pc: numerical taxonomy and multivariate analysis system. Version 2.1. Exeter Software, New York.

Scarano MT, Abbate L, Ferrante S, Lucretti S, et al. (2002). ISSR-PCR technique: a useful method for characterizing new allotetraploid somatic hybrids of mandarin. *Plant Cell Rep.* 20: 1162-1166.

Silva MC, Lemos RNS, Lima LHC, Gourlart Filho LR, et al. (2009). Variabilidade genética de *Bemisia tabaci* (Gennadius) biótipo B (Hemiptera: Aleyrodidae) em cultivos olerícolas em São Luís, MA. *Neotrop. Entomol.* 38: 762-768.

Silva DMP, Cavalcanti VALB, Sena RC and Arruda GP (2010). Controle Biológico de Praga: Considerações e Aplicabilidade em Pernambuco. In: Biotecnologia aplicada à agricultura: textos de apoio e protocolos experimentais (Figueiredo MVB, Burity HA, Oliveira J de P, Santos CE de R, et al., eds.). Embrapa, Brasília e Instituto Agronômico de Pernambuco, Recife.

Souza GA, Carvalho MRO, Martins ER, Guedes RNC, et al. (2008). Diversidade genética estimada com marcadores ISSR em populações brasileiras de *Zabrotes subfasciatus*. *Pesq. Agropec. Bras.* 43: 843-849.

von Dohlen CD and Moran NA (1995). Molecular phylogeny of the Homoptera: a paraphyletic taxon. *J. Mol. Evol.* 41: 211-223.

Walton VM and Pringle KL (2004). Vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), a Key Pest in South African vineyards. A Review. *S. Afr. J. Enol. Vitic.* 25: 54-62.

Wang X, Felker P, Burow DM and Paterson D (1998). Comparison of RAPD marker patterns to morphological and physiological data in the classification of *Opuntia* accessions. *J. Prof. Assoc. Cactus Dev.* 3: 3-14.

Warumby JF, Arruda Filho GP, Cavalcanti VALB and Arruda GP (2005). Pragas da Palma. In: A Palma no Nordeste do Brasil. 1st edn. (Menezes RSC, Simões DA and Sampaio EVSB, eds.). Recife: UFPE, Editora Universitária, Recife, 65-80.

