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Short Communication

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Partial Characterization of a Potyvirus Infecting African Oil Palm in South America

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With 5 figures

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Abstract

A virus with filamentous particles approximately 750 nm long was isolated from African oil palm (*Elaeis guineensis*) with 'chlorotic ring' leaf symptoms in south-western Colombia. The virus was mechanically transmitted from oil palm to brachiaria, maize, sorghum, and sugarcane (*Gramineae*) but not to any of 20 species in other plant families tested. In African oil palm, the virus is associated with cytoplasmic inclusions (pinwheels, laminated aggregates and scrolls) characteristic of subdivision III of the *Potyviridae*. Partial sequences obtained for fragments of the large nuclear inclusion (NIb) and capsid (CP) proteins, and for the entire 3′ untranslated region (UTR), showed amino acid sequence identities of 91.8% (NIb) and 95% (CP), and a nucleotide sequence identity of 90.8% (UTR) with strains of *Sugarcane mosaic virus* (SCMV). We thus conclude that the virus associated with the 'chlorotic ring' of African oil palm in Colombia is a species of the *Potyvirus* genus and, more specifically, a strain of SCMV.

Introduction

The *Potyviridae* is the largest family of plant viruses, which includes more than 180 virus species known to cause disease in over 60 families and 1800 plant species (Edwardson and Christie, 1991; Regenmortel et al., 2000). However, only one tentative potyvirus species, Palm mosaic virus (PalMV), infects a plant species in the family *Palmaceae*. PalMV causes mosaic, and ring and line patterns in ornamental Mexican fan palms (*Washingtonia robusta*) in California (Mayhew and Tidwell, 1978), and induces cylindrical (pinwheel) inclusions characteristic of subdivision II of the *Potyvirus* genus (Edwardson and Christie, 1991) in infected plant cells. Similar filamentous virus particles had previously been detected in symptomless African oil palms (*Elaeis guineensis*) from Papua New Guinea, but their viral nature was not confirmed (Plumb and Dabek, 1978).

In 1993, a potyvirus was isolated from Cuban royal palm (*Roystonea regia*) affected by chlorotic ringspot leaf symptoms in Queensland, Australia (Thomas et al., 1993). This virus was transmitted mechanically to plant species in the *Chenopodiaceae, Solanaceae* and *Papilionaceae*, and by the aphid *Myzus persicae* to *Nicotiana benthamiana*, and it induced cytoplasmic inclusions (pinwheels and laminated aggregates) typical of those of subdivision II of the *Potyviridae*. In 1994, a potyvirus detected in African oil palm cultivated in Karnataka, India, was associated with mosaic, mottling and ringspot leaf symptoms in young palms maintained in nurseries. Solomon and Babu (1998) observed that this potyvirus affected reproductive oil palm material imported into India from Costa Rica, Central America, and suggested the possibility of seed transmission. In 1995, a new disease of oil palm consisting of mottling, chlorotic streaks, and ringspots, was observed in the north-western oil palm production region of Ecuador. The causal agent was shown to be a potyvirus, which also induced the formation of cytoplasmic inclusions of subdivision II, consisting predominantly of laminated aggregates (Chinchilla et al., 1995). The new disease, named 'chlorotic ring', affected young palms between the age of 5 and 8 months. It was later suggested that this virus was probably Palm mosaic virus (Rivera et al., 1996).

Colombia, the first producer of African oil palm in Latin America, has four different agroecological zones planted to this crop. However, the south-western oil palm-producing zone located in the municipality of Tumaco, Department of Nariño, is the only region where diseases of apparent viral nature have been observed in Colombia (Morales, 1998). The first disease

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observed in this zone, in 1985, was 'sudden yellowing', later referred to as 'ringspot' (Jiménez, 1988, Jiménez and Peña, 1988). Although filamentous particles have been consistently observed in young palms affected by 'ringspot', preliminary results suggest that this disease is not caused by a potyvirus (F.J. Morales, unpublished information). The second viral-like disease of oil palm in south-western Colombia was detected in 1996 (Morales, 1999). This disease, unlike 'ringspot', was not lethal to affected oil palms, but it induced very conspicuous foliar symptoms consisting of eye-shaped lesions, predominantly on 5–8-month-old-palms in the nursery stage. This investigation was conducted to characterize the causal agent of this new disease of oil palm in Colombia.

Materials and Methods

Systemically infected, 9-month-old African oil palm seedlings (ASD, Costa Rica) showing ring-shaped foliar lesions (Fig. 1),which were used as sources of inoculum, were collected in Tumaco, Nariño, Colombia. All laboratory tests were conducted at the Virology Research Unit of the International Centre for Tropical Agriculture (CIAT), Palmira, Valle, Colombia. More than 15 African oil palms showing similar symptoms in south-western Colombia, were also tested to confirm the results presented here.

Pathogenicity tests

Infected plant extracts (1 : 4, w/v) prepared from symptomatic oil palm leaves in 0.1 M KPO_4 buffer, pH 7.6, were manually inoculated to the following species: *Avena sativa* cv. Clintland, *Brachiaria brizantha* CIAT accession 26646, *Chenopodium amaranticolor*, *C. murale*, *C. quinoa*, *Cucumis sativus*, *Datura stramonium*, *Glycine max* cv. Mandarin and Clark, *Nicotiana benthamiana*, *N. tabacum* cv. Samsun, *Panicum maximum* CIAT accessions 673, 6172 and 6299, *Sorghum bicolor* cvs. Atlas, Rio, Texas 2786 and Trudex, *Phaseolus vulgaris* cv. Bountiful, *Saccharum officinarum* accession CP-57–603, *S. halepense*, *Vigna unguiculata* cv. Blackeye, *and Zea mays* cv. Sikuanis. An average of five plants of each genotype were inoculated.

Fig. 1 Ring-shaped lesions on African oil palm leaves affected by 'chlorotic ring'

Electron microscopy

Leaf extracts from symptomatic African oil palms were negatively stained in 2% uranyl acetate, pH 3.7, and examined for the presence of virus particles using a JEOL JEM-1010 electron microscope (JEOL Ltd., Peabody, MA, USA). Leaf tissue of diseased African oil palms, was prepared for cytopathological examination as described earlier (Morales et al., 1990). Thin sections were cut with a diamond knife using a MT 6000 Sorvall ultramicrotome (DuPont Co., Newton, CT, USA).

Virus isolation

Symptomatic leaf tissue (200 g) harvested from either 11-month-old African oil palms (ASD) or 2-week-old sorghum 'Rio' seedlings, was homogenized at 4100 ***g*** for 5 min in a blender with four volumes per gram of plant tissue of 0.5 M KPO_4, pH 7.5, containing 0.5% Na_2SO_3 and 0.5 ml of chloroform and carbon tetrachloride. The mixture was passed through a glasswool filter; stirred with 6% PEG (polyethylene glycol) for 150 min at 5°C, and then centrifuged at 10 400 ***g*** for 35 min. The pellet was resuspended in 1/10th its original volume with 0.25 M KPO_4, pH 7.5, and then centrifuged at 3000 ***g*** for 5 min. The resulting supernatant was subjected to high speed centrifugation at 103 000 ***g*** for 2 h. The pellet obtained was resuspended in 0.25 M KPO_4, pH 7.5 and clarified at 1100 ***g*** for 5 min. The suspension was then layered onto preformed (26–40%) CsCl gradients and centrifuged for 2 h at 149 600 ***g***. The virus was recovered from the gradients and concentrated by centrifugation at 103 000 ***g*** for 2 h, and then re-suspended in 0.5 ml of 0.01 M KPO_4, pH 7.5.

Serology

A commercial monoclonal antibody capable of detecting a large number of potyviruses (Agdia, Inc., Elkhart, IN, USA) was used in indirect enzyme-linked immunosorbent assay (ELISA) (Voller et al., 1979) tests with leaf extracts of healthy and virus-infected palms.

Molecular cloning and sequencing

Viral RNA was extracted from purified virus according to the instructions provided with the RNeasy Kit (Qiagen, Valencia, CA, USA). Complementary (c) DNA synthesis was performed using 200 ng of random primers (Promega, Madison, WI, USA) in 50 μl of 50 mM Tris HCl, pH 8.3; 75 nM $MgCl_2$; 10 mM DTT (di-thiothreitol); 1 mM dCTP; 1 mM dGTP; 1 mM dTTP; 0.3 mM dATP; 30 μCi [α^{32} d ATP] ($\cong$ 3000 Ci/mmol), 60 units RNasin, 7 μg viral RNA, 600 U SuperScript II RT (Gibco BRL, Rockville, MD, USA) and incubated for 1.5 h at 37°C.

To synthesize the second cDNA chain, the reaction volume used for first cDNA synthesis was diluted to a final concentration of 50 mM Tris HCl, pH 7.2, 100 mM KCl, 3 mM $MgCl_2$, 3 mM DTT, 50 μg BSA (bovine serum albumin), 20 U DNA Polymerase I (Gibco BRL),

2 U RNase H (Promega), and incubated for 4 h at 14°C. The reaction was stopped by heating at 70°C, for 10 min, and subsequent cooling on ice.

For the fill-in reaction, 10 U of T4 DNA polymerase and a mixture of 3 mM dNTPs were added, and subsequently incubated for 15 min at 37°C. The enzyme was inactivated by adding 3 µl of 0.5 M EDTA. The cDNA was fractionated in a Sephacryl S-400 (Amersham Pharmacia Biotech, Piscataway, NJ, USA) column, and then ligated in de-phosphorylated Blue Script KS(+), digested with the enzyme Sma I (Promega).

Two clones of 2.2 kb and 276 b, were obtained from the above reactions. The larger clone was subcloned for partial sequencing. Sequence analysis was performed using an automatic sequencer (Perkin Elmer, Branchburg, NJ, USA) and BLAST program (NCBI, Bethesda, MD, USA). Phylogenetic analyses were conducted using DNAMAN for Windows (Lynnon Biosoft, Vaudreueil, Quebec, Canada).

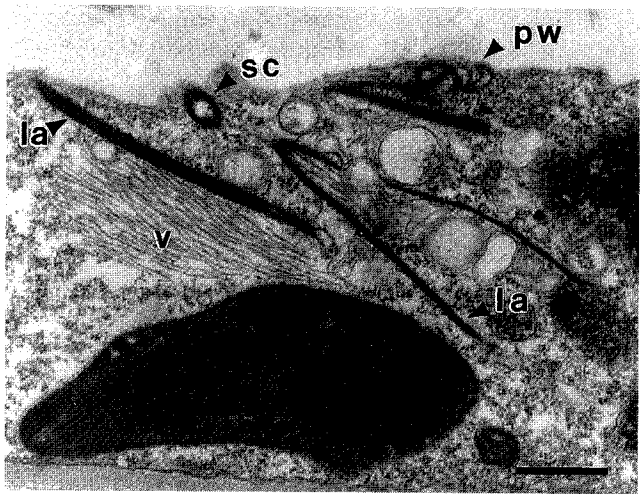

Fig. 3 Cylindrical inclusions: pinwheel (pw), scroll (sc), laminated aggregate (la), and virus-like particles (v) observed in leaf cells of African oil palm affected by 'chlorotic ring'. Bar = 500 nm

Results

Of the 24 plant genotypes inoculated, only four species of *Gramineae*, namely *B. brizantha* (brachiaria), *S. officinarum* (sugarcane), *S. bicolor* (sorghum) cvs. Rio and Trudex, and *Z. mays* (maize), developed mosaic symptoms and were shown by electron microscopy to contain flexuous filamentous particles approximately 750 nm in length and 15 nm in diameter. These particles were also observed in all foliar samples taken from young palms affected by 'chlorotic ring' (Fig. 2). The cytological examination of affected African oil palm foliar tissue revealed the presence of cytoplasmic inclusions consisting of pinwheels, laminated aggregates and scrolls, often associated with filamentous virus-like particles (Fig. 3). The purification protocol described above resulted in the isolation of filamentous particles similar to those observed in leaf extracts of oil palms affected by 'chlorotic ring' (Fig. 4). The yield of purified virus was approximately 50 mg/kg of infected leaf tissue

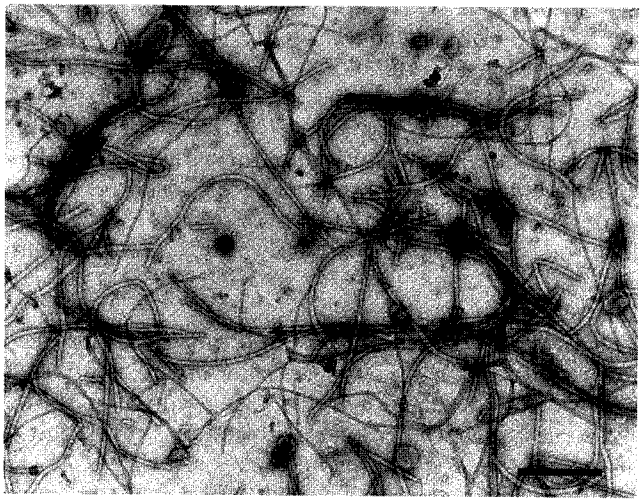

Fig. 4 Purified virus particles associated with the 'chlorotic ring' disease of African oil palm. Bar = 250 nm

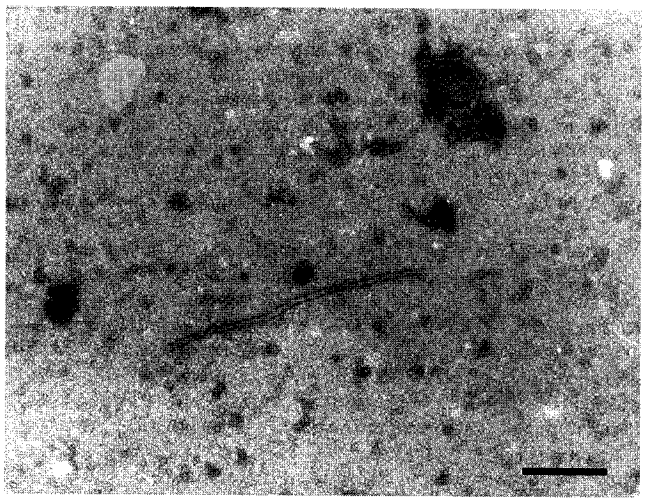

Fig. 2 Filamentous particle in leaf extract of diseased African oil palm. Bar = 200 nm

(uncorrected for light scattering) regardless of the propagation host. Diseased oil palm tissue reacted positively in indirect ELISA with the monoclonal antipotyvirus antibody. Six plants showing characteristic 'chlorotic ring' symptoms gave $OD_{405\ nm}$ values ranging between 0.144 and 0.949. Virus-free African oil palms used as controls, yielded $OD_{405\ nm}$ values within a range of 0.075–0.095.

Two subclones obtained from the 2.2 kb clone mentioned above for the African oil palm virus, yielded sequences corresponding to the putative large nuclear inclusion protein (NIb) found in *Potyvirus* genomes (Regenmortel et al., 2000). The 276 b clone was shown to contain sequences corresponding to the putative 3′ untranslated region (3′ UTR) and capsid protein (CP) of potyviruses. Comparative analyses of the amino acid (aa) sequences of the larger (553 bp) NIb subclone sequenced (GenBank acc. AY072882), revealed aa identities of 91.8, 80, 18.5 and 13% with the corresponding

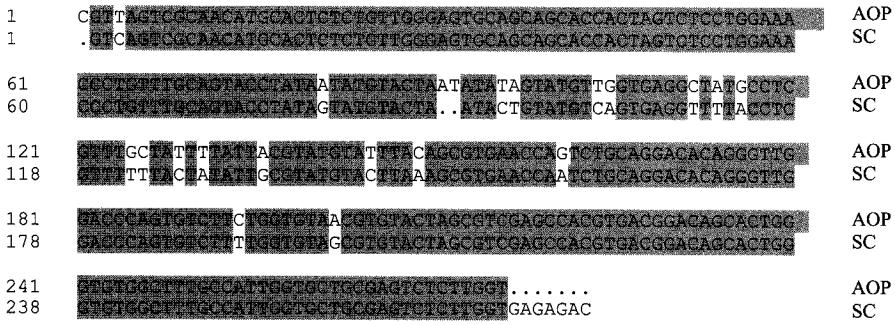

Fig. 5 Comparative sequence alignment of the 3′ untranslated regions of a potyvirus isolated from African oil palm (AOP) affected by 'chlorotic ring', and the sugar cane (SC) strain of *Sugarcane mosaic virus*

NIb segment of *Sugarcane mosaic virus* (SCMV) strain A (Acc.U84579), *Maize dwarf mosaic virus* (MDMV) Bulgarian isolate (Acc. AJ001691), *Johnsongrass mosaic virus* (JGMV) strain KS1 (Acc. U07218) and *Sorghum mosaic virus* (SrMV) strain H (Acc. U57358), respectively. Optimal alignments (Fig. 5) of 232 nucleotides of the 3′ UTR yielded nucleotide sequence identities of 90.8 and 93% between the African oil palm virus (Acc. AY072881) and SCMV strains A (Acc.U84579) and SC (Acc. D00948), respectively. Nucleotide sequence identities of 61.4, 60 and 45.1% were obtained for the 3′UTR of MDMV-Bulgaria, SrMV-H and JGMV-KS1, respectively, when compared to the corresponding region of the African oil palm potyvirus. The limited amino acid sequence obtained for the small (45 nucleotides) CP fragment attached to the UTR, showed identities greater than 95% with various SCMV strains.

Discussion

The present results showed that the 'chlorotic ring' disease of oil palm in south-western Colombia is consistently associated with the presence of a virus with filamentous particles belonging to subdivision III of the *Potyvirus* genus (Edwardson and Christie, 1991). The presence of subdivision I and II potyviruses affecting African oil palm in India, Mexican fan palm in the United States, and Cuban royal palm in Australia suggests the existence of different potyviruses infecting species of *Palmaceae*. The tentative characterization of the potyvirus that induces similar 'chlorotic ring' symptoms in Ecuador as Palm mosaic virus (Chinchilla et al., 1995; Rivera et al., 1996) is probably the result of insufficient cytopathological observations. The identification of the potyvirus associated with the 'chlorotic ring' disease of oil palm in south-western Colombia as a strain of *Sugarcane mosaic virus* (SCMV-AOP) is consistent with both the host range observed in this investigation, and the observation of subdivision III cytoplasmic inclusions in plant tissue infected by strains of SCMV (Lesemann et al., 1992).

The high nucleotide sequence identity (> 90%) observed for the NIb, 3′ UTR and CP segments of the oil palm virus genome and selected SCMV strains, further supports the identification of the African oil palm potyvirus found in southern Colombia as a strain of SCMV (Frenkel et al., 1989), hereafter referred to as SCMV-AOP. This finding makes the control of the virus difficult, given the large number of grass species susceptible to SCMV, and the different aphid species capable of transmitting this virus in a non-persistent manner (Persley, 1996). The current practice of replacing wild grasses in oil palm plantations by cover legume crops, and the isolation of oil palm plantings from gramineous crops (e.g. corn, sugarcane, sorghum), should reduce the incidence of SCMV-AOP in African oil palm nurseries and plantations.

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Literature

Chinchilla, C., C. Rivera, L. Moreira, R. Pereira (1995): Síntomas asociados a virus en viveros de palma aceitera en Ecuador. Informe Especial ASD, Costa Rica.

Edwardson, J. R., R. G. Christie (1991): The Potyvirus Group, Vol. I. Monograph no. 16-I. Agric. Exp. Sta., IFAS, University of Florida, Gainesville, FL, USA

Frenkel, M. J., C. W. Ward, D. D. Shukla (1989): The use of 3′ non-coding nucleotide sequences in the taxonomy of potyviruses: application to watermelon mosaic virus 2 and soybean mosaic virus-N. J. Gen. Virol. **70,** 2775–2783.

Jiménez, O. D. (1988): Mancha anular de la palma africana de aceite (*Elaeis guinensiis* Jacq.). Ascolfi Informa **14,** 55–56.

Jiménez, O. D., E. A. Peña (1988): Amarillamiento sorpresivo delas hojas jóvenes de la palma africana. Ascolfi Informa **14,** 21–24.

Lesemann, D.-E., D. D. Shukla, M. Tosic, W. Huth (1992): Differentiation of the four viruses of the sugarcane mosaic virus subgroup based on cytopathology. Arch. Virol. Suppl. **5,** 353–361.

Mayhew, D. E., T. E. Tidwell (1978): Palm mosaic. Plant Dis. Reptr **62,** 803–806.

Morales, F. J. (1998): La mancha anular de la palma de aceite: Avances de Investigación. Ceniavances no. 53. CENIPALMA, Bogotá, Colombia.

Morales, F. J. (1999): Investigación sobre la posible etiología viral de la 'mancha anular' de la palma de aceite en Colombia. Informe técnico final. CENIPALMA, Bogotá, Colombia.

Morales, F. J., A. I. Niessen, B. C. Ramirez, M. Castaño (1990): Isolation and partial characterization of a geminivirus causing bean dwarf mosaic. Phytopathology **80,** 96–101.

Persley, D. M. (1996): Sugarcane mosaic *potyvirus*. In: Brunt, A., K. Crabtree, M. Dallwitz, A. Gibbs, L. Watson (eds), Viruses of Plants, pp. 1204–1207. CAB International, University Press, Cambridge, UK.

Plumb, R. T., A. J. Dabek (1978): Viruses in oil palm. Trop. Agric. **55,** 59–63.

Regenmortel, M. H. V. van, C. M. Fauquet, D. H. L. Bishop, E. B. Carstens, M. K. Estens, S. M. Lemon, J. Maniloff, M. A. Mayo, D. J. McGeoch, C. R. Pringle, R. B. Wickner (2000): Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, New York, USA

Rivera, C., R. Pereira, L. Moreira, C. Chinchilla (1996): Detection of potyvirus-like particles associated with oil palms (*Elaeis guineensis*) in Ecuador. Plant Dis. **80,** 1301.

Solomon, J. J., M. K. Babu (1998): Incidence of potyvirus disease in oil palm nursery seedlings. J. Oil Palm Res. **10,** 52–56.

Thomas, J. E., A. F. Kessling, M. N. Pearson, J. W. Randles (1993): A potyvirus isolated from Cuban royal palm (*Roystonea regia*) in Queensland, Australia. Plant Pathol. **22,** 68–71.

Voller, A., D. E. Bidwell, A. Bartlett (1979): The Enzyme-Linked Immunosorbent Assay (ELISA). Dynatech Laboratories, Inc, Alexandria, VA, USA