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Short Communication

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Detection of Cucumber Mosaic Virus in Peanut (*Arachis hypogaea* L.) in Argentina

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Abstract

Peanut or groundnut (*Arachis hypogaea* L.) is an important oilseed crop in Argentina. Virus-like symptoms were observed in this crop during the 2000 growing season. Infected plants contained isometric particles *c*. 30 nm in diameter and tissue extracts reacted with a polyclonal antiserum to cucumber mosaic virus (CMV). A restriction profile typical of subgroup II was obtained for *Msp*I cleavage of cDNA produced by using specific primers to CMV capsid protein (CP). These results were also confirmed by nucleotide sequence data on the CP gene. This is the first report of CMV subgroup II infecting peanut.

Introduction

Arachis hypogaea L. (peanut) is the third most important oilseed crop in Argentina, one of the world's main peanut exporting countries. Peanut plants in fields in the mid-southern region of Córdoba (principal peanut-growing area) had virus-like symptoms of severe stunting, shortened petioles, malformed and small leaflets with chlorotic mottling (Fig. 1), severe reduction of the root system and the number and size of pods. Disease incidence in peanut fields ranged from 27% to 90%, and the yield of infected plants was severely reduced (March et al., 2000). The virus that was isolated was experimentally transmitted in a non-persistent manner by the aphid *Myzus persicae* (33–73% efficiency) (de Breuil et al., 2002). We have identified the cause of the disease by electron microscopic, serological and molecular techniques. A preliminary report has been published (Lenardon et al., 2000).

Materials and Methods

Collection and maintenance of virus isolate

Peanut plants showing characteristic symptoms were collected from fields in Alejandro Roca, province of Córdoba, Argentina, during the growing season. *Chenopodium quinoa* Willd. plants were mechanically inoculated with extracts from symptomatic peanut plants. The virus from a local lesion of *C. quinoa* was subcultured and maintained in *Nicotiana glutinosa* L. and *A. hypogaea*, which were retained in a growth chamber. This isolate was used in all our experiments.

Electron microscopy

Leaf dip preparations were made from symptomatic leaves, cut several times with a razor blade in a drop of sodium phosphate (0.01 M pH 7.5) containing 0.01% (w/v) sodium sulphite (Na_2SO_3). The leaf extracts were then transferred to carbon-coated collodion grids for 10 min. After washing with distilled water, they were negatively stained with 2% uranyl acetate and examined with a JEOL 1200 (Jeol, Tokyo, Japan) transmission electron microscope.

Serological tests

Leaf tissue from plants inoculated with the viral isolate were tested for cucumber mosaic virus (CMV) by using double antibody sandwich-enzyme-linked immunosorbent assay (Clark and Adams, 1977). Leaf samples were ground (1 g/10 ml) in extraction buffer (phosphate-buffered saline pH 7 + 0.05% Tween-20 + 2% polyvinyl pyrrolidone). Polyclonal and monoclonal CMV antisera (anti CMV, and anti-CMV subgroups I and II) (Agdia Inc., Elkhart, IN, USA) were used. Positive (Agdia Inc.) and negative (healthy peanut plants) controls were used in each assay. A_{405nm} values greater than the mean of the healthy controls plus three times the standard deviation were considered positive reactions.

Identification of isolate subgroup

An antigen-capture (AC) (Rowhani et al., 1995) followed by reverse transcription-polymerase chain

Fig. 1 Peanut plant showing (a) severe stunting and (b) reduced leaflets with chlorotic mottling

reaction (RT-PCR) was performed to confirm the presence of CMV in the plants that were tested. RT-PCR was performed with two CMV-specific primers that flank the CMV capsid protein (CP) gene (Rizos et al., 1992) using the Access RT-PCR System (Promega Corp. Madison, WI, USA). The following cycling parameters were used: 48°C for 45 min, 94°C for 4 min (40 cycles of 94°C for 30 s, 40°C for 30 s, 68°C for 1 min), and a final extension of 68°C for 7 min. The amplification products were assessed by electrophoresis in 1.4% agarose and stained with ethidium bromide. The cDNA that was obtained was then digested for subgroup identification (Rizos et al., 1992). *Msp*I restriction digestions were performed using 5 U of enzyme (Promega Corp.) with 18 μl of PCR-amplified reaction in a final volume of 30 μl. Digestions were carried out at 37°C for 4 h in the buffers supplied by the manufacturer, products were electrophoresed in acrylamide gels (10%) and stained with ethidium bromide.

cDNA cloning and sequencing

The cDNAs obtained by AC-RT-PCR were incubated at 72°C for 15 min with 2.5 U of AmpliTaq DNA polymerase (Promega Corp.). The cDNAs were cloned using the TOPO TA Cloning Kit for Sequencing (Invitrogen Corp., San Diego, CA, USA). The recombinant clones containing the CP gene were purified using the Plasmid Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and prepared for sequencing according to the recommendations of the Biotechnology Resource Center (DNA Services, Cornell University, NY, USA). CMV CP sequences were compared with those from other CMV strains available at the National Center for Biotechnology Information (NCBI) web site: http://www.ncbi.nlm.nih.gov. Multiple sequence alignment was performed using the CLUSTAL W option of the MegAlign program (Lasergene software, DNASTAR, 2001); identity analysis of deduced amino acid sequences was performed.

Results

Isometric particles *c*. 30 nm in diameter with dark-stained centres characteristic of cucumoviruses were seen by electron microscopy. Infected plant tissues reacted strongly with polyclonal antiserum to CMV whereas serological tests for CMV subgrouping failed to provide conclusive results (data not shown). A cDNA fragment of *c*. 870 bp was amplified from the samples that were tested (Fig. 2). *Msp*I digestion of PCR products yielded four fragments of approximately 250, 200, 160 and 130 bp (Fig. 3). This pattern allowed the classification of the isolate within subgroup II. Six clones from the peanut isolate were selected for sequencing the complete CP gene. The isolate, designated as CMV-Pe, had a high degree of nucleotide sequence identity (97.4–98.2%) with the known CMV subgroup II strains and $<77.3\%$ identity with the CMV subgroup I strains. Amino acid identity comparisons of the entire CMV-Pe CP with other CMV strains revealed a homology between 97.7% and 99.5% for CMV subgroup II members and between 77.1% and 79.4% for members of subgroup I (Table 1).

These results indicate that CMV infects peanut crops in the main cultivated area of this oilseed crop in Argentina and that the CMV isolate belongs to subgroup II.

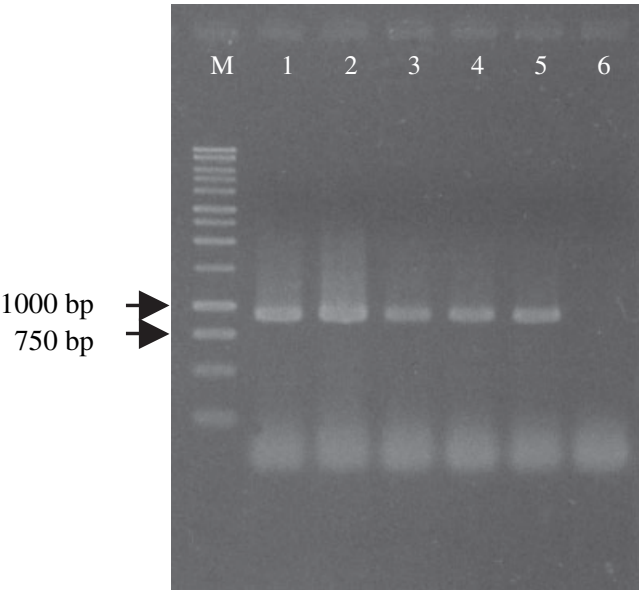

Fig. 2 Analysis of RT-PCR-amplified CMV capsid protein cDNAs. M: 1 kb DNA Ladder marker (Promega); lanes 1–5, leaf extracts of peanut plants with symptoms; lane 6, negative control (healthy peanut)

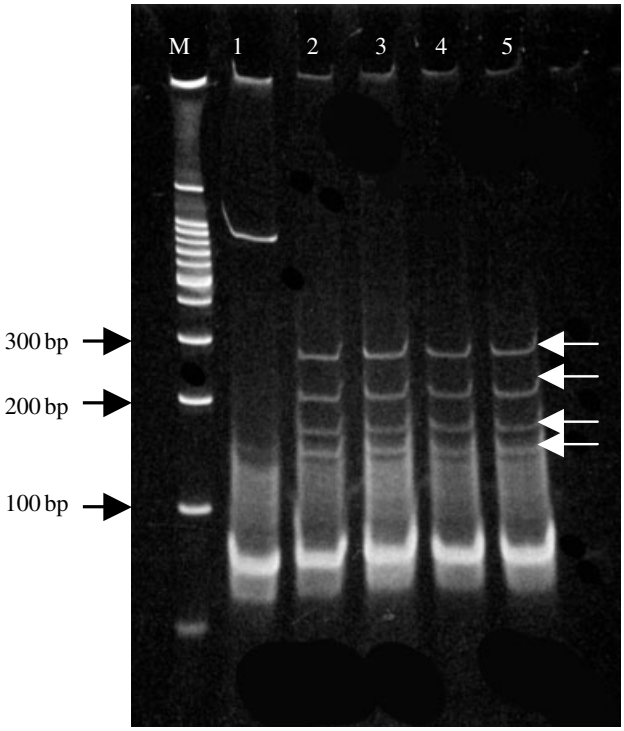

Fig. 3 *Msp*I restriction analysis of RT-PCR-amplified CMV products. M: 100 bp DNA Ladder marker (Promega); lane 1, non-digested RT-PCR product; the remaining lanes correspond to *Msp*I digests

Table 1
Percentage amino acid homology among the capsid protein of the peanut isolate of CMV (CMV-Pe) and other CMV strains

Strains	Q	WL	S	LS	Fny	D	M	Ny	CS
CMV-Pe	99.5	98.6	97.7	99.5	78.9	79.4	77.1	78.9	79.4
Q		98.2	97.2	99.1	79.4	79.8	77.5	79.4	79.8
WL			96.3	99.1	78.4	78.9	76.6	78.4	78.9
S				97.2	78.0	78.4	76.1	78.0	78.4
LS					78.4	78.9	76.6	78.4	78.9
Fny						99.5	96.8	100	98.6
D							96.3	99.5	98.2
M								96.8	95.4
Ny									98.6
CS									

Discussion

In the present work, the aetiological agent of a disease in peanuts was identified as CMV (genus cucumovirus, family Bromoviridae). Strains of CMV can be divided into subgroups I and II on the basis of their similarity in sequence and serological relationship (Palukaitis et al., 1992). Surveys of naturally infected crops suggest that subgroup I strains occur more frequently than those of subgroup II (Crescenzi et al., 1993).

The natural occurrence of CMV in peanut has been reported only from China (Xu and Barnett, 1984), where peanut is infected by two CMV strains, a strain of minor importance, CMV-CS, and the predominant strain, CMV-CA; both strains belong to subgroup I (Kokalis-Burelle et al., 2000). The CMV-Pe isolate found in Córdoba, Argentina, is a member of subgroup II. Interestingly, white clover plants (*Trifolium repens* L.) growing in peanut fields and exhibiting symptoms similar to those in peanut were also shown to be infected with CMV-Pe, whereas the CMV-CA strain failed to infect this species (Xu and Barnett, 1984). This is the first report of CMV infecting peanut in Argentina and of CMV subgroup II occurring in peanut. The occurrence of CMV in peanuts is possibly associated with the spread of peanut cultivation to southern, more temperate areas, where the virus probably is present in other crops.

Cucumber mosaic virus is one of the most economically important plant viruses infecting some hundreds of plant species, including many vegetables and ornamentals (Douine et al., 1979). It has the potential to remain in a region for long periods, even in the absence of crops, because of its wide host range among perennial weed species (Gillaspie et al., 1998). CMV is readily transmitted by more than 80 aphid species during brief probes, as well as by mechanical transmission and by seed (Gallitelli, 2000). Studies of the epidemiology of CMV and the identification of virus-resistant peanut genotypes have been initiated to devise strategies to control the virus.

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References

de Breuil S, Giolitti F, Nome C, Lenardon SL. Evidencias de *Cucumber mosaic virus* en cultivos de maní. XI Jornadas Fitosanitarias Argentinas, 26–28 de junio de 2002, Córdoba, Argentina, Río Cuarto, 2002, p. 73.

Clark ME, Adams AN. (1977) Characteristics of microplate methods of enzyme linked immunosorbent assay for detection of plant viruses. *J Gen Virol* **34**:475–483.

Crescenzi A, Barbarossa L, Gallitelli D, Martelli GP. (1993) Cucumber mosaic cucumovirus populations in Italy under natural epidemic conditions and after a satellite-mediated protection test. *Plant Dis* **77**:28–33.

DNASTAR. *Lasergene Biocomputing Software for Windows*, Ver. 5. Madison, WI, USA, DNASTAR, 2001.

Douine L, Quiot JB, Marchoux G, Archange P. (1979) Recensement d'espèces végétales sensibles au virus de la mosaïque du cocombre (CMV). Étude bibliographique. *Ann Phytopathol* **11**:439–475.

Gallitelli D. (2000) The ecology of *Cucumber mosaic virus* and sustainable agriculture. *Virus Res* **71**:9–21.

Gillaspie AG, Hajimorad MR, Ghabrial SA. (1998) Characterization of a severe strain of *Cucumber mosaic cucumovirus* seedborne in cowpea. *Plant Dis* **82**:419–422.

Kokalis-Burelle N, Porter DM, Rodríguez-Kábana R, Smith DH, Subrahmanyam P. *Compendium of Peanut Diseases*, 2nd edn. St Paul, MN, USA, APS Press, 2000, pp. 57–58.

Lenardon SL, Giolitti F, Dujovny G, Marinelli A, March GJ. Asociación de partículas tipo *cucumovirus* en maní con síntomas

de severo achaparramiento. XV Jornada Nacional de Maní, 14 de setiembre de 2000, Córdoba, Argentina, Gral Cabrera, 2000, pp. 32–33.

March GJ, Marinelli A, Lenardon SL et al. Incidencia, distribución espacial y pérdidas por *cucumovirus* en maní. XV Jornada Nacional de Maní, 14 de setiembre de 2000, Gral Cabrera, Córdoba, Argentina, 2000, p. 34.

Palukaitis P, Roossinck MJ, Dietzgen RG, Francki RIB. (1992) Cucumber mosaic virus. *Adv Virus Res* **41**:281–348.

Rizos H, Gunn LV, Pares RD, Gillings MR. (1992) Differentiation of Cucumber mosaic virus isolates using the polymerase chain reaction. *J Gen Virol* **73**:2099–2103.

Rowhani A, Maningas MA, Lile L, Daubert SD, Golino DA. (1995) Development of a detection system for viruses of woody plants based on PCR analysis of immobilized virions. *Phytopathology* **85**:347–352.

Xu Z, Barnett OW. (1984) Identification of a Cucumber mosaic virus strain from naturally infected peanuts in China. *Plant Dis* **68**:386–389.