

Detection and characterization of a phytoplasma associated with frog skin disease in cassava

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Abstract

Cassava is one of the main carbohydrate sources in the tropics, and it has worldwide importance as basic food for millions of persons in America and Africa. This root crop is affected by cassava frog skin disease that in Colombia produces yield losses of almost 90%. A phytoplasma was associated with this disease by using a nested-PCR followed by RFLP analyses, cloning and sequencing of 16S ribosomal gene. Sequence analysis showed that the phytoplasma was related to those belonging to 16SrIII ribosomal group, with a 99% sequence homology. Evidence of phytoplasma aetiology for the disease was achieved also by electron microscopy observations, grafting to specific host plants, and by its dodder transmission. Symptoms were reproduced in healthy plants. Symptoms disappearance in leaves was obtained by growing cassava infected plantlets in a chloroxytetracycline solution producing further evidence for the phytoplasma aetiology. This study is the first to report a phytoplasma associated with cassava frog skin disease.

Key words: Plant disease, aetiology, phytoplasmas, molecular identification, PCR/RFLP, sequencing.

Introduction

Cassava (*Manihot esculenta* Crantz) is one of the main carbohydrate sources in the tropics and it has worldwide importance as basic food for millions of persons in America and Africa.

This root crop is affected by cassava frog skin disease (CFSD), a major disease of roots that is spreading throughout the Colombian cassava-growing areas, where yield losses reach almost 90%, and other South American countries such as Brazil, Venezuela and Panama. The major symptom is that severely affected roots are thin and not useful for consumption.

Considering the epidemic behaviour of the disease and its economic importance a study was carried out to verify possibility of phytoplasma aetiology of CFSD disease in Colombia.

Materials and methods

Cassava samples collected from diverse tissues of symptomatic and asymptomatic plants were tested after nucleic acid extraction by nested PCR assays with three pairs of universal primers P1/P7 (Deng and Hiruki, 1991; Schneider *et al.*, 1995), R16mF2/R1, and R16F2n/R2 (Gundersen and Lee, 1996), to amplify the 16S rRNA and 23S rRNA region of phytoplasmas. Amplicons of the correct size obtained after amplification with R16F2n/R2 were subjected to RFLP analyses with restriction enzymes *AluI*, *MseI*, *RsaI*, and *TaqI*. To further verify phytoplasma identity sequencing of R16F2n/R2 amplified DNA fragments was also carried out after their cloning.

Tissue preparation and embedding for electron microscope observation was performed from symptomatic

cassava tissues.

Graft transmission trials from symptomatic plants were carried out on *Secundina* spp., a foliar disease indicator variety of cassava.

Growing infected cassava plantlets in a chloroxytetracycline solution was also performed.

Results

Phytoplasmas were detected in CFSD infected cassava samples by using nested-PCR assay with the three primer pairs described above. Fragments measuring 1.2 kb were amplified only from samples collected from symptomatic plants. The detected phytoplasmas were firstly identified as related to X-disease phytoplasma ribosomal group after RFLP analyses on R16F2n/R2 amplicons.

Sequence analysis of the cloned fragments confirmed that the phytoplasma present in symptomatic cassava samples was similar to phytoplasmas belonging to 16SrIII ribosomal group (X-disease and related phytoplasmas) showing a 99% sequence homology with phytoplasmas in GenBank belonging to this ribosomal group.

After electron microscopy observations the presence of phytoplasmas was also observed in the phloem sieve tubes of roots from symptomatic plants. No phytoplasmas were detected in healthy cassava control tissues.

By grafting in *Secundina* spp. plants CFSD symptoms were reproduced. In addition, phytoplasmas were detected by nested-PCR after transmission by *Cuscuta* spp. (dodder plant) and grafting in two cassava varieties.

Symptoms disappeared from cassava leaves when infected plantlets were grown in a chloroxytetracycline solution.

Discussion

This study is the first to report a phytoplasma associated with cassava frog skin disease. Molecular identification allows referring the identified phytoplasmas to ribosomal group 16SrIII (Lee *et al.*, 1998).

Several experimental evidence suggests that phytoplasmas can play also an important pathogenetic role in CFSD aetiology, moreover during preliminary studies evidence of aerial vector presence was found. Further researches are in progress to better verify epidemiological aspects of this disease.

References

- DENG S., HIRUKI C., 1991.- Amplification of 16S rRNA genes from culturable and nonculturable *Mollicutes*.- *Journal of Microbiological Methods*, 14: 53-61.
- GUNDERSEN D. E., LEE I.-M., 1996.- Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs.- *Phytopathologia Mediterranea*, 35: 144-151.
- LEE I.-M., GUNDERSEN-RINDAL D. E., DAVIS R. E., BARTOSZYK I. M., 1998.- Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences.- *International Journal of Systematic Bacteriology*, 48: 1153-1169.
- SCHNEIDER B., SEEMÜLLER E., SMART C. D., KIRKPATRICK B. C., 1995.- Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas, pp. 369-380. In: *Molecular and Diagnostic Procedures in Mycoplasmaology*, vol. 2 (RAZIN S., TULLY J. G., Eds).- Academic Press, San Diego, CA, USA.

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