

Detection of phytoplasma infection in okra in Mauritius

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Abstract

Using PCR and RFLP techniques, 16SrXII and 16SrV phytoplasma groups were identified from okra (*Abelmoschus esculentus* Moench) leaf samples collected in Mauritius. The most frequent symptoms observed on okra plants were leaf yellowing, leaf distortion, curling and overall stunting. The phytoplasma infection was widely distributed across the island and a high incidence of 75 to 100% was recorded at inspected sites. This is the first report of the presence of phytoplasmas in okra in Mauritius.

Key words: Phytoplasmas, PCR/RFLP, okra, plant disease.

Introduction

Okra (*Abelmoschus esculentus* Moench) known also as lady's fingers or gumbo is a flowering plant in the Malvaceae family. It is valued for its edible green seed pods. Originating from Africa, the plant is cultivated in tropical, subtropical and warm temperate regions around the world. In Mauritius, okra is commonly known as lalo and its green pod is a popular vegetable. Major diseases commonly seen on okra are: powdery mildew and fruit rot caused by *Choanephora*. Annual production amounts to around 1,275 T over an area of 170 ha (Anonymous, 2010).



Figure 1. Top: okra plant showing stunting, leaf yellowing and distortions; bottom: close up of symptomatic leaves.

(In colour at www.bulletinofinsectology.org)

To date, there is no record of the occurrence of phytoplasma disease on okra in Mauritius. Phytoplasmas have only been recently identified in Mauritius on tomato (Dookun *et al.*, 1999; Gungoosingh-Bunwaree *et al.*, 2007), watercress and onion (Gungoosingh-Bunwaree *et al.*, 2010).

Since December 2010, okra growers across Mauritius reported a new disorder with unusual symptoms on the two local okra varieties. The most frequent symptoms comprised leaf yellowing, leaf distortions, upward leaf curling and overall stunting (figure 1).

A very high incidence of disorder ranging from 75 to 100% was encountered at the 36 okra planting sites visited across the country. A yield loss of around 40% was reported by okra growers. A high infestation of *Amrasca biguttula* (Hemiptera: Cicadellidae) was also reported at plantations inspected.

Materials and methods

Fresh symptomatic and asymptomatic okra leaf samples from the north and south of the island were employed for nucleic acid extraction to verify phytoplasma presence.

Total nucleic acids were extracted from 1 g of leaf midribs from symptomatic and asymptomatic okra leaves (Prince *et al.*, 1993), dissolved in Tris-EDTA pH 8 buffer, and maintained at 4°C. Forty ng/μl of nucleic acid were used for amplification. Samples employed as controls included DNA extracts from asymptomatic okra, STOL (stolbur from pepper from Serbia, 16SrXII-A), ULW (elm yellows from EU, 16SrV-A), GLAWC (gladiolus witches' broom from France, 16SrI-B) infected *Catharanthus roseus*. Tubes without DNA were used as no template controls. Direct PCR was carried out with primer pair P1/P7, followed by nested PCR with R16F2n/R2 (Gundersen and Lee, 1996) and with 16R_{758F}/16R_{1232R} (Gibb *et al.*, 1995).

After preliminary RFLP identification with *TruI* on amplicons obtained from the latter primers further nested PCR assays were carried out with primers

R16(V)F1/R1 and R16(I)F1/R1 (Lee *et al.*, 1995). PCR and nested PCR reactions were carried out following the protocol of Schaff *et al.* (1992).

Identification of detected phytoplasmas was done using RFLP analyses on 16R_{758F}/16R_{1232R} amplified ribosomal DNA fragments with *TruI*, *RsaI*, *HhaI*, *Tsp509I*, *TaqI*, *AluI* (Fermentas, MBI, Vilnius, Lithuania) restriction enzymes. Polyacrylamide 5% gels stained with ethidium bromide were employed to compare profiles to reference phytoplasmas (Bertaccini *et al.*, 2000).

Results

The 90% of symptomatic okra leaf sample tested positive to phytoplasma presence by using 16R_{758F}/16R_{1232R} primers in second nested PCR assays on R16F2/R2 amplicon size of about 500 bp. Asymptomatic negative controls and no template controls did not produce amplification. Preliminary RFLP analyses carried out with *TruI* restriction enzyme on these amplicons indicate that the profiles could be referable to phytoplasmas belonging to groups 16SrXII and 16SrV.

To further verify the phytoplasma identity, group specific primers were employed in nested PCR. Amplification with R16(I)F1/R1 primers allowed detection of phytoplasmas in all symptomatic samples tested and RFLP analyses with *TruI* confirmed that 16SrXII phytoplasmas are associated with the described symptoms. In some of the samples R16(V)F1/R1 primers provided amplification confirming the presence of 16SrV group phytoplasmas in mixed infection with 16SrXII phytoplasmas (stolbur).

Discussion

The above findings indicate that phytoplasmas are widening their host range in Mauritius. A phytoplasma of the 16SrV group was previously identified in tomato (Gungoosingh *et al.*, 2007), whilst a 16SrXII group phytoplasma was recently identified on onion and watercress in Mauritius (Gungoosingh *et al.*, 2010).

However it is the first time that these two phytoplasma groups are being identified in okra in Mauritius. Further studies need to be carried out in order to determine the correlation among yield loss, disease incidence, and role played by *Amrasca biguttula* in phytoplasma transmission across okra plantations and other crops in Mauritius.

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