The Detection and Molecular Characterization of a Phytoplasma Associated with *Machorreo* of Lulo (*Solanum quitoense*) in Colombia

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Introduction

Lulo or naranjilla (*Solanum quitoense*) is a fruit with commercial potential for food processing in Colombia. It is attacked by a disease known as *machorreo*, the causal agent of which has yet to be determined. The disease is a serious constraint to lulo production in the country, with reports of production having been reduced by as much as 80%. The disease characteristically stunts the plant and causes floral abortion. The objective of this study is to detect presence of phytoplasmas in lulo attacked by machorreo.

Figure 1. Lulo or naranjilla (*Solanum quitoense*)

Figure 2. Diseases symptoms caused by phytoplasma, include stunting and floral abortion.

Materials and Methods

PCR. DNA, isolated from symptomatic plants, was used to amplify 16S ribosomal DNA fragments by PCR, using two pairs of universal primers. The products were re-amplified by using nested PCR and employing other primers to detect and confirm that phytoplasmas were associated with *machorreo* (Table 1).

Table 1. Primers used in nested PCR, conducted to detect the presence of phytoplasmas in lulo attacked by the phylody and virescense disease machorreo.

First cycle	Second cycle	Approx. fragment size (base pairs)
P1/P7	R16F2N/R16R2	1200
R16mF2/R16mR1	R16F2N/R16R2	1200
LD16-1/23S	P4/P7	550
R16F2/R16R2	R16(III)F2/R16(III)R1	800

RFLP's. To identify their phytoplasma group on the basis of patterns of RFLPs, the nested-PCR products (1.2 kb) were digested with enzymes AluI, MseI, RsaI, and TaqI. Results were verified by re-amplifying R16F2/R16R2 products (0.8 kb), using a group-specific (16Sr III) primer pair.

rDNA sequences. The 1.2-kb and 0.8-kb fragments were cloned and sequenced. Analysis of 16S rDNA sequences determined that the phytoplasma was similar to GenBank accessions.

Transmission by grafting. We grafted buds and petioles from both diseased and healthy lulo plants onto plants of Catharanthus roseus cv. Vinca. The grafted plants were then kept in the greenhouses at ICA's Palmira Experiment Station until symptoms appeared.

Results and Discussion

Detection by PCR. Primers that gave the best results are showed in table 2. The last primers were specific to all the phytoplasmas of the 16Sr III group (X-disease) (Figure 3). The expected sizes in base pairs were obtained for each primer in the lulo samples and the vinca and coffee controls. The primers of the X-disease group amplified only for the phytoplasmas obtained from lulo and the coffee control. The vinca controls did not amplify for this region, suggesting an approximation to the group to which the lulo phytoplasma would belong.

Figure 3. Amplifications with nested PCR, using specific primers. Lanes 1, 1-kb marker; lanes 2–5 = lulo phytoplasma sampled from different village districts, Colombia; lane 6 = coffee phytoplasma; lane 7 = healthy lulo plant; lane 8 = vinca phytoplasma; lane 9 = negative control.

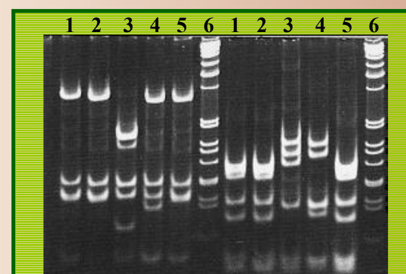

Figure 4. RFLPs with enzymes *AluI* and *MseI*, fragments amplified by nested PCR with primers R16F2N/R16R2. Lanes 1-2 = lulo; lane 3 = 16Sr I group (palm phytoplasma); lane 4 = 16Sr IX group (vinca phytoplasma); lane 5 = 16Sr III group (coffee phytoplasma); lane 6 = 1-kb Marker.

Restriction fragment length polymorphisms (RFLPs). The lulo phytoplasma was placed in the 16Sr III group (Lee et al. 1993) (Figure 4).

Sequence analysis. The phytoplasma analyzed in this study presented high levels (97%) of homology with the sequences of the 16Sr III group (X-disease). (Table 3)

Transmission by grafting indicated that the disease is contagious. The *C. roseus* cv. Vinca plants, grafted with buds and petioles from diseased lulo plants, showed yellowing of leaves, noticeably reduced leaf size, and diminished plant development. These symptoms are similar to those described for diseases caused by phytoplasmas (Oshima et al. 2001). The Vinca plants grafted with tissues from healthy lulo plants had no symptoms.

Table 2. Primers used in nested PCR, conducted to detect the presence of phytoplasmas in lulo attacked by the phylody and virescense disease machorreo

Primers	Positive per symptomatic sample (%)	Reproducibility (%)
P1/P7 and R16F2N/R16R2	100	75
R16mF2/R16mR1 and R16F2N/R16R2	100	75
LD16-1/23S and P4/P7	100	80
R16F2/R16R2 and R16(III)F2/R1 (specific)	100	100

Table 3. The degree of homology found between sequences of phytoplasma DNA obtained, through nested PCR, from lulo attacked by machorreo and sequences of phytoplasmas reported in NCBI's GenBank. [a] Number of homologized bases in different regions of the sequence reported in GenBank.

Pairing with GenBank	GenBank code	Homologized bases[a]	Homology (%)
Chayote witches'-broom phytoplasma (ChWBIII)	AF147706	1004/1031	97
Milkweed yellows phytoplasma	AF510724	1003/1031	97
Blueberry proliferation phytoplasma	AY034090	1002/1031	97

Conclusion

This study is the first to report a phytoplasma associated with *machorreo* in lulo. We detected the presence of a phytoplasma in association with *machorreo* in lulo, using phylogenetic analysis and homology with the sequence of the 16S rRNA region and the gene tRNA. We identified the phytoplasma as belonging to the 16Sr III group (X-disease; 97% homology). For future studies, we recommend identifying possible vectors through samplings of crop insects, particularly of the order Homoptera. Through molecular techniques, crop weeds should also be evaluated for their potential as hosts to the phytoplasma. Finally, as a possible management practice, resistance of promising lulo hybrids to *machorreo* should also be evaluated.

References

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