Phytoplasmas as Causal Agents of Celosia Disease in Israel

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Abstract. Recently, yellows diseases have become more common in Israel, and phytoplasmas have been detected in some of these diseased crops. Commercial fields of two celosia species (Celosia plumosa L. and C. cristata L.) also have exhibited yellows symptoms and total crop failure. Typical mycoplasma-like bodies were observed in infected but not in healthy plants. The same plants were analyzed for the presence of phytoplasma by polymerase chain reaction (PCR), using the universal oligonucleotide pair r16SF2/r16SR2, followed by nested PCR using group-specific primers. Restriction analyses performed with these products indicated that two different types of phytoplasmas are infecting celosia. PCR-RFLP analysis of one type revealed a restriction pattern typical of aster yellows. Similar analysis of the second type indicated possible relatedness, though not identity, to the pattern of phytoplasmas of the Western-X group. This is, to our knowledge, the first report of phytoplasma infection in celosia.
DNA extraction. Extracts were prepared from leaves, hairy roots and main roots as described by Maixner, et al. (1995). Fresh or frozen tissue (0.5–1.0 g) was ground in 5–7 mL of extraction buffer (100 mM Tris-HCl pH 8.0, 2% cetyl trimethyl ammonium bromide (CTAB), 1.4 M NaCl, 20 mM EDTA, 0.2% β-mercaptoethanol). The slurry was incubated for 20 min at 60 °C, and centrifuged (10 min, 3200 × g). The supernatant fluid was collected and extracted with an equal volume of chloroform : isooamyl alcohol (24:1 v/v) and precipitated with 1 volume of isopropanol. Following 20 min at −20 °C, the preparation was centrifuged at 14,900 × g for 20 min. The pellet was washed twice with 70% ethanol, dried and resuspended in 50 µL distilled water.

Polymerase chain reaction (PCR). The universal phytoplasma primer pair P1/P7 (Schneider et al., 1993) amplifies a 1800 bp-long fragment that extends from the 5’ end of the 16S rDNA gene to the 5’ region of the 23S DNA. This primer pair was used for phytoplasma detection in DNA extracts of the tested plant tissues. The P1/P7 PCR product was further amplified by nested PCR with the U5/U3 primer pair (Lorenz et al., 1995). Another universal primer pair, r16SF2/r16SR2 was synthesized according to published sequences (Lee et al., 1993), and used to amplify a 1.2-kbp fragment of the conserved region of the 16S rRNA of all known phytoplasmas. In this case, the PCR was made directly with the r16SF2/r16SR2 primer pair, followed by a nested PCR with group-specific primers for AY and CX that were also-synthesized according to Lee et al. (1994) and Lee and Davis (1998). The primer pair STOL11 f2/f1, amplifying a stolbur-specific 1.7 kbp-long fragment, was synthesized according to Daire et al. (1997) and used for PCR. The DNA template in all PCR assays was 20 ng in a 30-µL assay. DNA was heated to 94 °C for 5 min, after which Taq polymerase was added, and the mixtures were subjected to 35 PCR cycles (92 °C, 30 s; 55 °C, 30 s; 72 °C, 50 s). The PCR program ended with a 7-min elongation step at 72 °C.

The DNA extracted from symptom-free plants and that extracted from greenhouse-grown plants served as negative controls in PCR. DNA extracted from periwinkles identified as carrying AY-type, CX-type and Stolbur-type phytoplasma, served as positive controls.

RFLP analysis of PCR products. The PCR products were analyzed by restriction endonuclease digestion, using MsiI, AluI, and KpnI. The DNA was digested (37 °C; 20 h) in the specific buffers corresponding to the enzymes. Fragments were separated by electrophoresis on 5% polyacrylamide gels, followed by staining with ethidium bromide as described above. The positive controls were DNA extracts from the aforementioned phytoplasma-infected periwinkle plants.

Results and Discussion

A typical infected celosia is illustrated in Fig. 1. Test plants, mechanically inoculated with sap from infected celosia, did not exhibit any symptoms related to viral infection, indicating that no mechanically transmissible virus was associated with the disease. Electron microscope observations of crude plant extracts revealed no virus or virus-like particles (not shown).

The observation of typical phytoplasma bodies in thin sections of phloem sieve tubes from infected celosia plants (Fig. 2), and their absence from the corresponding symptom-free plants, indicated that a phytoplasma could have been the causative agent of the disease. No viral particles could be detected in these thin sections, which supports the aforementioned mechanical inoculation tests in suggesting that no viral infection was involved.

Use of the universal primers P1/P7 indicated that all celosia plants showing symptoms were infected with phytoplasma, while all symptom-free plants were phytoplasma-free (data not shown). Group-specific PCR assays, using the universal 16SF2/16SR2 primer pair and followed by nested PCR with group-specific primers, indicated the involvement of AY in celosia infections in most of the infected plants (Fig. 3A). This was corroborated by restriction analysis both of the PCR product of the nested AY-specific bands (Fig. 3 B and C) and of the P1/P7 bands (data not shown). However, a few infected plants produced amplification products with the CX-specific primers. The resultant products were somewhat longer than that of the CX-positive control (Fig. 4A), and the restriction analysis indicated a pattern similar but not identical to that of the control CX band (Fig. 4B). Negative results were obtained with the Stolbur primers (data not shown). We propose a new type of phytoplasma resembling CX and the AY type of phytoplasma as the causative agents of celosia yellows disease in Israel. Double infection, in which a plant carried both phytoplasma types, was not observed.

In recent years, several types of phytoplasma have been identified in various crops,
such as grape (*Vitis vinifera* L., Tanne and Orenstein, 1997) and carrot (*Daucus carota* L., Orenstein et al., 1999) in Israel. The aster yellows type along with the Western-X and Stolbur types is the most prevalent, and mixed infections sometimes occur. Phytoplasma has been associated with disease symptoms in a few ornamental plants, such as *Anemone coronaria* L., *Eustoma russellianum* L., and *Lilium longiflorum* L. (Cohen et al., 1999), but no severe damage has been recorded.

### Summary

The objective of this study was to determine the causative agent of a yellows-type disease that causes severe damage to celosia. Molecular analysis and electron microscopy demonstrated that the disease was caused by phytoplasmas. Two different types of phytoplasma could be detected in the diseased plants (but not in healthy ones), one belonging to the AY group and the other, closely related, but not identical, to the CX group. The same CX-resembling type has also been detected in carrots in Israel (Orenstein et al., 1999). The characterization of this phytoplasma and the search for the possible vector(s) are currently in progress.

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