

Natural occurrence of phytoplasma associated with chickpea phyllody disease in Pakistan – a new record

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During spring 2005–06 chickpea plants (cvs Desi and Kabuli) were found to be affected by a previously undescribed disease in Pakistan. Symptoms consisted of proliferation of branches with smaller leaflets, giving a bushy appearance to the plants. Affected plants were scattered in the field and were more easily spotted at flowering and podding time. The flowers developed abnormal green structures (phyllody) instead of normal flowers. At the time of crop maturity when the healthy plants were drying the diseased plants in the field were conspicuously green.

Tissue samples from plants with and without symptoms were examined using a transmission electron microscope to ascertain if the disease was associated with a phytoplasma. Typical pleomorphic bodies (phytoplasma) mostly spherical to oval of a size ranging from 200–600 nm were observed only in the sieve elements of affected samples. The bodies had opaque, low electron density cytoplasm that contained ribosome like granules, DNA-strand-like structures and lacked nuclear membranes, similar to previously reported phytoplasmas (Ajayakumar *et al.*, 2007). Such bodies were absent from healthy samples, from xylem cells, phloem parenchyma cells and companion cells of affected plants. In addition, DNA was extracted from plants with symptoms and amplified using the universal phytoplasma PCR primers P1/P7 followed by R16F2n/R16R2. PCR products were characterized by RFLP analysis and partial sequencing. All these samples gave a 1250 bp PCR product and the RFLP profile associated with 16SrII phytoplasmas, and partial sequencing confirmed that the phytoplasma had the greatest homology to 16SrII phytoplasmas. Koch's postulates were followed using 5-week-old chickpea plants grown

in pots inoculated by grafting. A set of control plants was kept without grafting. Phyllody symptoms similar to those observed in the field started to develop after 25–35 days in graft-inoculated plants while no disease symptoms were observed on control plants.

Chickpea has been grown in Pakistan for many years without any reported incidence of phyllody. The presence of phytoplasma disease in chickpea presents a new threat to the chickpea in Pakistan. Previously the disease was found in Ethiopia, India, Myanmar (Ghanekar *et al.*, 1988); Australia (Saqib *et al.*, 2005) and Oman (Al-Saady *et al.*, 2006). This is the first report of chickpea phyllody disease in Pakistan.

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First report on the association of a 16SrII phytoplasma with sesame phyllody in Pakistan

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Sesame phyllody disease has been recorded on sesame in Pakistan for a number of years, and is characterized by virescence, phyllody, yellowing, floral sterility and stem proliferation of infected plants. The disease causes significant losses in Pakistan (Sarwar *et al.*, 2006) but prior to this report the causal agent had not been identified. However, in Oman, phytoplasmas of the 16SrII group have been reported as the causal agent of sesame phyllody (Al-Sakeiti *et al.*, 2005).

Tissue samples from infected and uninfected plants were examined using a light microscope using Dienes' stain. Regularly distributed dark blue areas were observed in the phloem cells of stem, leaf and stalk sections of infected sesame plants but these areas were absent from phloem cells of healthy samples, confirming that a phytoplasma is associated with the disease in sesame. To identify the phytoplasma associated with the disease, DNA was extracted from affected plants and amplified using the universal phytoplasma PCR primers P1/P7 (Deng & Hiruki, 1991) followed by R16F2n/R16R2 (Gundersen & Lee, 1996). All samples from infected plants gave a 1250 bp PCR product and the RFLP profile associated with 16SrII phytoplasmas when digested with *Hae*III, *Rsa*I and *Alu*I and compared with Faba bean phyllody (GenBank Acc. No. EF193355) and Australian tomato big bud (Acc. No. EF193359). Partial sequencing (500 bp from each end using primers R16F2n and R16R2) confirmed that the phytoplasma had > 99% sequence identity with sesame phyllody from Oman (Acc. No. EU072505). The disease was shown to be graft transmissible

in a greenhouse experiment. Six week-old sesame plants grown in pots were inoculated by grafting. A set of control plants was kept without grafting. Phyllody symptoms similar to those observed in the field started to develop after 50–60 days in the graft-inoculated plants whilst no disease symptoms were observed on control plants.

This is the first molecular evidence for the association of a phytoplasma of the 16SrII group with phyllody disease in Pakistan and its sequence is essentially identical to that of the phytoplasma causing sesame phyllody in Oman.

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