Microscopic studies of phytoplasma causing sesame phyllody

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Abstract
Sesame (Sesamum indicum L.) is one of the major and valuable oilseed crops in the world because of its high oil quality. Phyllody incited by mycoplasma-like organisms (MLO) is one of the considerable diseases of sesame. This pathogen is present in most of the sesame production area in the world and causes important yield losses. In the present study investigations were carried out on the microscopic studies of phytoplasma. Light microscopy of hand cut sections treated with Dienes’ stain showed blue areas in the phloem region of phyllody-infected plants. Pleomorphic bodies (phytoplasma structures) were observed in phloem sieve elements in diseased plants using transmission electron microscopy (TEM).

Keywords: Dienes stain, transmission electron microscopy, light microscopy

Introduction
Sesame (Sesamum indicum L.) is an important oilseed crop grown in tropics and subtropics and it is also known by “queen of oil seeds”. Sesame seed is a rich source of protein (20%) and edible oil (50%), and contains about 47% oleic acid and 39% linolenic acid. Phytoplasmas are non-helical obligate parasites that belong to the prokaryotic class Mollecutes and are transmitted by sap-feeding insects and vegetative plant propagation materials. The symptoms starts with vein clearing of leaves. The disease manifests itself mostly during flowering stage, when the floral parts are transformed into green leafy structures, which grow profusely. The flower is rendered sterile. The other disease symptoms are floral virescence, proliferation, seed capsule cracking, formation of dark exudates on foliage and floral parts, and yellowing. Sesame phyllody is transmitted by a leafhopper (Orosius albicinctus). Phytoplasmas are able to move within plants through the phloem from source to sink and they are able to pass through sieve tube elements. Phytoplamas are pleomorphic and have small genome. In plants, they are restricted to the phloem tissue and spread throughout the plant by moving through the pores of the sieve plates which divide the phloem sieve tubes. Plants infected by phytoplasmas exhibit a wide range of specific and non-specific symptoms. Symptoms of diseased plants may vary with the phytoplasma, host plant, stage of the disease, age of the plant at the time of infection and environmental conditions.

Materials and Methods
To study sesame phyllody under light microscopy and electron microscopy

Light microscopy
Light microscopy studies were carried out as per the procedure of Neinhau et al., 1982 [9] and Deeley et al., 1979 [3]. Leaves were collected from sesame plants which exhibited symptoms of phyllody (Plate 1) and from healthy plants without symptoms (Plate 2) grown in IIOR field. Free hand sections of ten pieces of each from healthy and infected leaves were taken in 1-2mm size with a razor blade. The leaf pieces were kept in petriplates and fixed using glutaraldehyde solution for 2 days at 40°C. The sections were stained with 0.2% Dienes stain at 30°C. The stained sections were observed under compound microscope 10X.

Transmission electron microscopy
Phytoplasma is not cultivated in nutrients media, the methods of its detection and diagnosis are restricted. So electron microscopy is used to check the presence of phytoplasma units in phloem tissues of infected plants.
Water agar-embedded healthy and phyllody infected sesame plant samples were pre-fixed in 5% glutaraldehyde overnight, washed with 0.2 M Pipes buffer, and post fixed in 1% osmium tetroxide for 18 h at room temperature. The samples were washed with distilled water, treated with 5% uranyl acetate for 16-18 h, and washed again with distilled water. These were then dehydrated with absolute ethanol and embedded in Spur resin over a period of 1-2 days to achieve maximum resin infiltration. The samples were polymerized in pure Spur resin in moulds incubated at 70 °C for 48 h. The polymerized resin blocks were hand trimmed and ultra-thin serial sections 120 nm thick were cut on an RMC MT 7000 ultra-microtome. The sections were put on copper grids, and double stained with 5% uranyl acetate for 30 min and lead citrate for 10 min. Observations were made at Ruska labs, Rajendranagar, Hyderabad, with a transmission electron microscope operating at 80 kV.

**Results and Discussion**

**Light microscopy**

Thin hand cut sections of phyllody-infected and healthy leaves when treated with Diene’s stain and observed under light microscope showed blue areas in the phloem region. The blue area revealed the presence of phytoplasma in the phyllody infected leaves (Plate 3). However, the healthy leaf sections did not show any blue color though treated with Diene’s stain (Plate 4). Similar results were obtained by Salehi & Izadpanah (1992) [7] and Akhtar et al. (2009) [1].

**Transmission electron microscopy**

The presence of phytoplasma bodies in the ultrathin section of midrib was confirmed by Transmission Electron Microscope (TEM). The electron micrograph showed typical pleomorphic phytoplasma bodies inside the phloem cells of ultrathin section of the midribs (Plate 5). Examination of ultrathin sections from leaves of diseased sesame revealed the presence of numerous phytoplasma units in sieve tubes of phloem leaves but not in healthy leaves. Phytoplasmas were particularly abundant in mature sieve tubes. When present in low concentrations, phytoplasmas were generally restricted to the periphery of sieve tubes. EL-Banna et al., (2015) also observed pleomorphic bodies typical to phytoplasma structures in phloem sieve elements in ultrathin sections of infected plants using Transmission Electron Microscope (TEM). Similarly clusters of phytoplasma cells ranging from 300 -800nm in diameter in phloem sieve elements was observed by Tseng et al., (2014) [9], Choopanya (1973) [1], Sahambi and Prasad (1977) [6], Tamimi et al. (1989) [8] also reported electron micrograph of an ultrathin section sesamum phyllody phloem tissue showing the presence of numerous mycoplasma like organisms in the sieve elements.

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