Research Paper

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# First Report of the Natural Occurrence of Phyllody Disease of Sesame in Syria

Agriculture

**KEYWORDS :** Phytoplasma, Orosius albicinctus D, Sesame, Virescence, PCR

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## ABSTRACT

Survey of sesame fields in Hama province of Syria was done during June to October 2010, wherein most of the fields showed typical phyllody symptoms and the incidence was varied between (7-66 %). Different types of phyllody disease symptoms viz., floral virescence, phyllody, Witches broom, formation of dark exudates on floral parts and yellowing, shoot apex fasciation, and short internodes with twisted and reduced leaves were observed. Orosius albicinctus D. was found as the most predominant insect species in most infected sesame fields, which is known to play an important role in transmitting the phytoplasma. Dienes' stain of leaves and stems sections of sesame tissue plants revealed the presence of regularly distributed dark blue areas in the phloem cells of stem sections of infected plants, which was absent in the healthy plants. Total DNA was extracted from symptomatic and non-symptomatic tissues and direct PCR was carried out using universal primers P1/P6 followed by nested PCR with specific primers R16F2n/R16R2 for 16SrRNA gene. Amplification of 1800 and 1250 bp from the phyllody infected plants confirmed the phyllody disease in the infected samples of sesame which was absent in the healthy samples.

**Introduction:**

**Sesame (***Sesamum indicum L.), one of the oldest oilseeds cultivated in some provinces of Syria, has been known for its culinary and medicinal properties for centuries (Al-Matt and Blaukh, 1985). Sesame seed is rich in fat, protein, carbohydrates, fibre and edible oil (Shyuand Hwang, 2002). Pests and diseases is one of the major factors limiting the production and productivity of this crop in Syria. Phyllody disease caused by a phytoplasma is a very serious disease in several sesame growing regions of the world and decreases the yield, especially in warm climates (Salehi and Izadpanah, 1992). Phytoplasma are specialised bacteria that are obligate parasites of plant phloem tissue and transmitted through insects and causes disease in more than 700 plant species (Lee et al., 2000; IRPCM, 2004) and reduces the economic yield in low and high value crops worldwide (Bertaccini, 2007). Because of lake of information about phyllody disease in sesame crop from Syria, this study was aimed to demonstrate the first report of phytoplasma associated with phyllody disease of sesame. Here we describe the disease symptoms, light microscopy and molecular detection of phytoplasma associated with sesame phyllody in Syria based on 16S rDNA PCR detection.*

**Materials and methods:**

**Survey was conducted in the sesame fields in Hama province of Syria during June to October 2010. Samples of phyllody disease were collected from 26 sesame fields and disease percentage was calculated using formula (Per cent infection of plant observed = No. of Infected Plants per $M^2$ /** Total no. of Plants /per $M^2$)*100. Insect species were collected from the infected plants using sweep nets (100 sweeps). Light microscopy of leaves and stems of healthy and infected tissue sections were stained for 10 min in 0.2% solution of Dienes' stain as described by (Deeley et al., 1979). To confirm the phytoplasma infection, total DNA was extracted from symptomatic and non-symptomatic tissues. Direct PCR was carried out using universal primers P1 (5;-AAGAGTTTGATCCTGGCTCAG-GATT-3') and P6 (5'-GGTAGGGATACCTTGTTACGACT -3') (Deng and Hiruki, 1991) followed by nested PCR with specific primers R16F2n (5'- GAAACGACTGCTAAGACTGG-3') and R16R2 (5'-TGACGGGCGGTGTGTACAAACCCCG -3') for 16S rRNA gene as described by Gundersen and Lee (1996). DNA was extracted from healthy and infected sesame plants by a previously described method (Deng and Hiruki, 1991). One µl (20ng/µl) of extracted genomic DNAs were used as PCR templates for direct PCR and 1 µl of undiluted PCR product per sample was used in new tubes for the nested PCR. The PCR protocol used with the first round primers was as follows: 94°C for 2 min, 1cycle; 94°C for 30 sec, 60°C for 1 min, 72°C for 1 min 30 sec, 35 cycles; 72°C for 10 min as final extension .The cycling protocol for the second round was similar, but the annealing temperature was used 55°C instead of 60°C. PCR products were analysed by electrophoresis through 1 % agarose gel, stained with ethidium bromide, and DNA bands visualized using a UV transilluminator. Standard DNA fragment size 1 kb was used as ladder.

**Results and discussion:**

**Survey of the Sesame's field in Hama province of Syria revealed that most of the sesame plants showed phyllody symptoms. The phyllody incidence (7-66 %) was varied in relation the variety, plant age, temperature and insects present (Data not shown). Phytoplasma infected plants exhibited symptoms suggesting a profound disturbance in the normal balance of growth regulators and also yellowing symptoms (Bertaccini and Duduk, 2009). Different types of phyllody disease symptoms were observed on sesame plants viz.,** *floral virescence (Fig.1), phyllody (Fig.2), Witches broom (Fig.3), formation of dark exudates on floral parts and yellowing (Fig. 4), shoot apex fasciation, and short internodes with twisted and reduced leaves (Fig. 5). Symptoms of phyllody disease observed in Syria were similar to the symptoms previously reported from many countries like Pakistan, India, Iran and turkey (Akhtar et al., 2009).* Species of *Diptera, Hemiptera, Homoptera* and *Lepidoptera* have been identified on sesame crop. Among them *Orosius albicinctus D. was found as the most predominant species (Data not shown), which is known to play an important role in transmitting the phytoplasma pathogen which was proved by several authors (Akhtar et al., 2009; Esmailzadeh-Huosseini et al., 2007). Dienes' stain is widely used to detect the presence of phytoplasma in plant tissue (Deeleyet al., 1979). Dienes' stain of an examination of hand cut or freezing microtome sections of infected and uninfected tissue of sesame stems under light microscope revealed regularly distributed dark blue areas in the phloem cells of stem sections of infected plants (Fig. 6), but not in the healthy plants, confirming that the phytoplasma is associated with the disease in sesame, this results are with contrast with previous results reported by (Salehi and Izadpanah 1992; Akhtar et al., 2009). PCR technique using universal primers can be used for identifying different groups of phytoplasma (Gundersenand Lee, 1996; Akhtar et al., 2009). The direct and nested PCR with specific primers R16F2n/R16R2 for 16S rRNA gene amplified a product*

*size of 1800 and 1250 bp, from all infected samples and not in healthy samples (Fig. 7). From the above results of symptom observation, microscopy observation of infected plants, presence of insect vector Orosius albicinctus D., and specific amplification of DNA amplicon product of 1800 and 1250 bp from the infected plant we confirmed the association of phytoplasma with the phyllody disease of sesame. To our knowledge, this is a primary report of the natural occurrence of a phytoplasma pathogen associated with the phyllody disease of sesame in Syria.*

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**Fig. 1: Sesame inflorescence showing floral virescence.**

**Fig. 2: Sesame phyllody symptom.**

**Fig. 3: Witches broom symptom in the sesame plant.**

**Fig. 4: Yellowing and dark exudates on upper parts**

**Fig. 5: Shoot apex fasciation and presence of short internodes with twisted, reduced leaves**

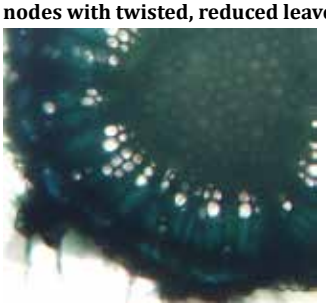

**Fig. 6: Transverse section of light microscopy of infected sesame stem treated with Dienes' stain showing dark blue areas in phloem region.**

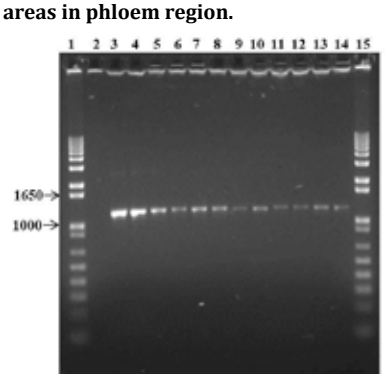


**Fig. 7: Nested PCR amplification of phytoplasma 16S rRNA gene from infected sesame plants using universal primer pair R16F2n/R16R2. Lanes 1 and 15, 1-Kb DNA ladder; Lane 2, healthy sample; lanes 3-14, infected samples. Amplified PCR products(1250 bp) were separated by 1% agarose gel electrophoresis.**

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