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Detection of Phytoplasma Diseases in Sesamum through PCR

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ABSTRACT

Phyllody is a destructive disease of sesamum, Phyllody, Floral virescence and bushy appearance are the most common symptoms produced by phytoplama. Phytoplamal infection from sesamum was detected by PCR technique. In the present investigation amplified product of expected size is 1.2 kb from phytoplasma infected samples by PCR by using primer pair with R16F2/R16R2 for specific for the 16S rDNA sequences of phytoplasma. The PCR based techniques are very much useful for eradication and produce pathogen free planting material of sesamum.

Key words: *rDNA, PCR based techniques, Sesamum, Phyllody*

INTRODUCTION

Sesame (*Sesamum indicum* Linnaeus) 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[8]. 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[4]. 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[1]. 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[2].

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Nucleic acid-based applications have been developed for the detection and identification of phytoplasmas in plants and vectors. Polymerase chain reaction (PCR) amplification of 16S rRNA with either phytoplasma species-specific primers or phytoplasma group-specific primers has been preferred widely for molecular diagnostics[7]. Phytoplasma detection is now routinely carried out by different nucleic acid techniques based on the polymerase chain reaction (PCR). These techniques are adequate for detecting phytoplasmas in both plant material and insect vectors. They are helping to prevent the spread of these diseases and reduce their economic impact.

MATERIALS AND METHODS

Symptomology and Collection of infected sample

Phytoplasma infected samples were collected from sesamum field, department of biotechnology, Kakatiya University. Infected plants are showed characteristic symptoms of witch's broom, bushy appearance. Under field condition, infected plants showed the most common characteristic symptom is phyllody. The samples were kept in plastic bags with labels indicating date of collection and brought to laboratory for further assay.

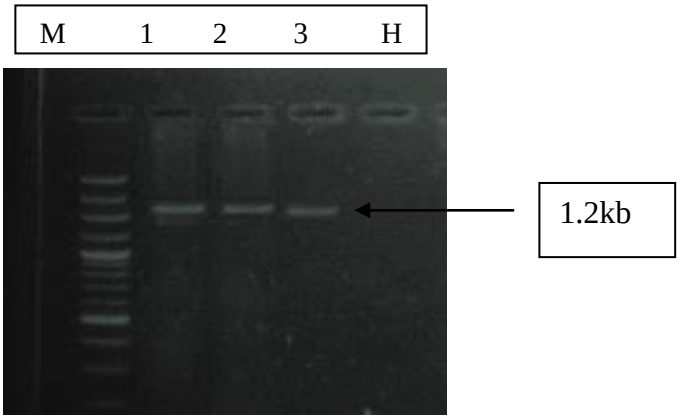
Extraction of DNA and PCR (Polymerase chain)

Total DNA from phytoplasma infected samples were isolated by using DNeasy Plant Mini Kit (QIAGEN) according to manufacturing protocol. The extracted DNA use as template for PCR and PCR was carried out with various parameters such as run with PCR using phytoplasma specific universal primer set -Forward primer R16F2, 5'-ACGACTGCTGCTAAGACTGG-3' and Reverseprimer R16R2,5' TGACGGGCGG TGTGTACAAACCCCG-3'. PCR master mix was prepared with total of 20 µl reaction volume containing 2 µl PCR Reaction Buffer, 10X without MgCl2 5µl;2 µl MgCl2 25mM, 0.5 µl; dNTPs 10mM each, 2µl of Primer-forward and -reverse 10mM each , 5 Unit µl-1 of Tag DNA polymerase and final volume mae up with Double distilled sterile water, and,. PCR programmes for amplification of DNA is 94°C for 4min for initial denaturation, 45 sec for denaturation; 58°C for 1 min for annealing; 72°C for 2 min for extension and 10 min for final extension for total of 30 cycles. The Amplified products were analyzed by electrophoresis using 1% agarose gel and were visualized in a Gel documentation unit.alongside 1kb plus DNA ladder as molecular weight marker.

RESULTS AND DISCUSSION

In the present investigation amplified product of expected size is 1.2 kb from phytoplasma infected samples by PCR by using primer pair with R16F2/R16R2 for specific for the 16S rDNA sequences of phytoplasma, while no amplicon was obtained from healthy samples of sesamum. The total DNA was extracted from phytoplasma infected sesamum samples by DNeasy Plant Mini Kit (QIAGEN) according to manufacturing protocol. The PCR program was performed by using following parameters for amplification of DNA is 94°C for 4min for initial denaturation, 45 sec for denaturation; 58°C for 1 min for annealing; 72°C for 2 min for extension and 10 min for final extension for total of 30 cycles. The PCR products were analyzed by electrophoresis using 1% agarose gel .alongside 1kb plus DNA ladder as molecular weight marker and were visualized in a Gel documentation unit. Phytoplasma infected plants showed stunted growth, witches broom and leaf yellowing and the abnormal excessive development of buds leading to bushy appearance with small leaves. Most important characteristic symptoms of the phytoplasma is phyllody. Phytoplasma infected sesamum samples were collected from fields of the department of biotechnology. The samples were kept in plastic bags with labels indicating date of collection and brought to laboratory for diagnosis assay. The typical symptoms shown by phytoplasma infected plants include: whitening, yellowing or reddening of the leaves indicating chlorosis, shortening of the internodes leading to stunted growth, smaller

leaves and excessive proliferation of shoots resulting in a 'broom' phenotype, loss of apical dominance and phylloidy[2] . The similar symptoms of phytoplasma also reported by Ragimekula *et al*[5]. In PCR assay all the symptomatic sesamum plant are showed positive reaction to phytoplama. The PCR based techniques are very much useful for detection of phytoplasma in sesamum. The similar results earlier reported by using phytoplasma specific primer R16F2n/R16R2 and produce a PCR product of 1.2 kb on phytoplasma infected celosia argentea and perennial fruit tree[3,6].



M-ladder, 1, 2, 3 infected samples showed positive reaction in PCR and H-Healthy samples

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