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Symptomatology, transmission and molecular detection of phytoplasma infecting Brinjal (*Solanum melongena* L.)

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Abstract

Brinjal or eggplant (Solanum melongena L.) is an important and widely consumed indigenous vegetable crop of India grown round the year. Among the diseases, Brinjal Little leaf (BLL) is one of the most important economic diseases caused by phytoplasma, belonging to the class mollicutes, under which wall less prokaryotic organisms are grouped. The diseased plants showed characteristic symptoms including little, narrow, soft, glabrous and smooth leaves produced as clusters, phyllody, proliferation of axillary shoots along with shortened internodes, stunted growth etc. Graft inoculation was 100% successful and initial symptoms were noticed in the new sprouts of the rootstock 7-10 days after the graft transmission. The molecular detection was done through PCR and it produced an amplification of ~1.8 kb with the primer P1/P7.

Keywords: Brinjal little leaf (BLL), Phytoplasma, Mollicutes, Phyllody, Graft transmission and PCR.

I. INTRODUCTION

Phytoplasmas are specialized bacteria that are obligate parasites of plant phloem tissue and named mycoplasma-like organisms or MLOs. They are characterized by their lack of cell wall, pleiomorphic or filamentous shape, normally with a diameter less than 1 µm, and their very small genomes. The organism was discovered in ultrathin sections of plant phloem tissue with yellows symptom [4].

A common symptom caused by phytoplasma infection is phyllody, the production of leaf like structures in place of flowers. Plants infected by phytoplasma exhibit an array of symptoms that suggests profound disturbances in the normal balance of growth regulators. Symptoms include virescence/phyllody, sterility of flowers, proliferation of axillary (side) buds resulting in witches ' broom appearance, abnormal internodes elongation and generalized stunting [2]. The phytoplasma can be transmitted through insect vectors, dodders and grafting. The highly conserved 16S rRNA gene sequence has been used as the primary molecular tool for the detection and classification of phytoplasmas.

II. MATERIALS AND METHODS

A. Symptomatology

Brinjal plants with little leaf and phyllody symptoms were collected from the Crop museum, College of Agriculture, Vellayani. The various symptoms produced on brinjal plants inoculated with phytoplasma were recorded and the culture was maintained by the repeated grafting of infected scion onto healthy root stocks.

B. Transmission

The infected shoots having the symptoms of phytoplasma were selected as the source or scion. The base of the scion was trimmed into a wedge shaped structure of approximately 4cm and then it was inserted into the cleft of about 4 to 5cm made on the healthy plant which is selected as the root stock. Then the graft was tied firmly using a high density polythene strip and covered using a polypropylene cover to keep the union moist. The grafted plants were kept for the expression of symptoms on the new sprouts.

C. Molecular detection

C.1. Isolation of genomic DNA

The genomic DNA was isolated using QIAGEN plant minikit as per the manufacture's instruction.

C.2. Confirmation of genomic DNA

The presence of genomic DNA was confirmed by the horizontal gel electrophoresis using 0.8% agarose gel made of 1x TAE buffer and ethidium bromide (0.5µg/ml). The DNA samples were mixed with the gel loading dye and added to the wells prepared. Run the electrophoresis unit till the loading dye reaches about 3/4th of the gel. After the completion of electrophoresis, the gel was visualized with the help of gel documentation unit (BIORAD).

C.3. PCR

PCR was performed by using the primer P1/P7 (Table.1) under different thermal conditions (Table.2). All the PCR operations were performed in a Thermal cycler. PCR was performed in 15µl reaction mixture consisting of the following:

10x Taq buffer (with MgCl2)	: 1.5µl
10mM dNTP	: 0.6 µl
Taq polymerase (1unit/ µl)	: 1 µl
Forward primer P1 (10pM)	: 0.5 µl
Reverse primer P7 (10pM)	: 0.5 µl
Template DNA	: 2µl
Sterile distilled water	: 8.9µl

Table.1 Primers Used In PCR Amplification.

Primer	Primer sequence	Reference
P1	**5' AAGAGTTTGATCCTGGCTCAGGATT 3'**	**[3] & [6]**
P7	**5' CGTCCTTCATCGGCTCTT 3'**	

Table.2 PCR Conditions

PCR Primer combination	Process	Temperature (°C)	Time	No. of cycles
P1/P7	**Initial denaturation**	**94**	**5 min**	**35**
	Denaturation	**94**	**45 sec**	
	Annealing	**63**	**1 min**	
	Extension	**72**	**2 min**	
	Final extension	**72**	**10 min**	

The PCR products were analyzed by using horizontal electrophoresis unit. Agarose gel (1%) was prepared with ethidium bromide (0.5µg/ml) and TAE/TBE buffer (running buffer). A marker (1Kb DNA ladder: 2µl) was mixed with 1µl of 6x dye and 3µl of sterile water and load to the well. The PCR products each of 5µl was mixed with 2µl of the dye and load to the appropriate wells. After the dye has reached 3/4th of the gel, it was visualized in the gel documentation system (BIORAD) and the amplification was analyzed.

III. RESULTS AND DISCUSSION

3.1 SYMPTOMATOLOGY

The graft inoculated brinjal plants showed characteristic little, narrow, soft, glabrous and smooth leaves produced as clusters and later showed the symptoms of yellowing. Newly formed leaves were much shorter and the petioles were shortened so that the leaves appeared to be sticking to the stem. The proliferation of axillary shoots along with shortened internodes and numerous clustered small leaves gave the plant a stunted bushy appearance. The typical symptom of phyllody, the production of leaf like structures in place of flowers was observed. The phylloid flowers are erect, green and mostly sterile. Fruiting was not observed. In India, the symptoms of little leaf disease have been recorded in aubergine (brinjal) [5].

Figure 1, 2 & 3. Phyllody in brinjal

Figure4: Little leaf in brinjal.

3.2 GRAFT TRANSMISSION

Graft transmission was carried out by wedge grafting of phytoplasma infected scion onto a healthy root stock and observed for the symptom development. The initial symptoms were noticed in the new sprouts of the rootstock, 7-10 days after the graft transmission. Successful graft transmission of phytoplasma has been reported in many plants. The development of little leaf symptoms forty days after the grafting of periwinkle plants was progressed towards young vegetative organs along with phyllody and virescence [7].

Figure5: Graft transmission in brinjal

3.3 MOLECULAR DETECTION

The genomic DNA was isolated and confirmed by the horizontal gel electrophoresis, visualized using the gel documentation system. A 1.8 kb fragment corresponding to the entire 16S rRNA gene along with the 16S-23S spacer region and the 5'end of the 23S rDNA was obtained in the direct PCR using the universal phytoplasma primers P1/P7. The results of the present study are in line with the previous reports in which, PCR products of ~1.8 kb were obtained in direct PCR with phytoplasma universal primer pair P1/P7 in brinjal samples and the vectors [1].

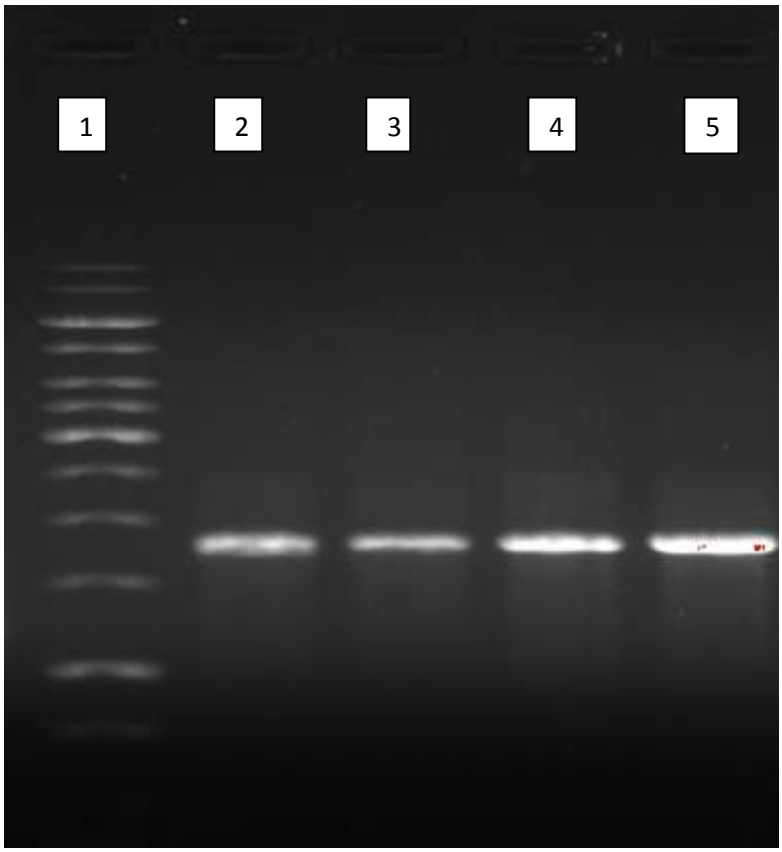

Figure 6: PCR products obtained using the primer pair P1/P7.

Product size: ~1.8 kb

Lane1: 1kb DNA Ladder

Lane 2-5: Brinjal samples from symptomatic plants.

IV. CONCLUSION

The phytoplasma infection in brinjal produces characteristic little leaf and Phyllody symptoms, which can be detected by PCR using the universal primer P1/P7. The grafting of infected scion onto healthy root stock gives 100% successful transmission of phytoplasma, if the grafting is properly done.

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