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Graft Transmission of Brinjal Little Leaf Disease Caused by *Candidatus* Phytoplasma Trifolii

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ABSTRACT

Brinjal little leaf disease is caused by Candidatus Phytoplasma trifolii. The disease is transmitted mainly by leaf hoppers and also transmitted by graft. The little leaf Infected plants of brinjal were used as donor or source plants for grafting. Phytoplasma was transmitted by wedge grafting from donor little leaf infected brinjal to receptor healthy brinjal plants and developed little leaf symptoms within 20-25 days after grafting. There is no little leaf symptoms development in graft failed plants and control plant that is healthy brinjal scion grafted to healthy brinjal rootstock. All the plants (graft successed and contol palnts) samples were subjected to PCR using primers P1/P7 universal primers specific to 16S rRNA gene of phytoplasmas. Which resulted in expected amplicons of size ~1·8 kb observed only in case of graft successes brinjal plants whereas no amplification in healthy control plant.

Key words: *Brinjal, Phytoplasmas, Virescence, Phyllody, Sterility*

INTRODUCTION

Brinjal (*Solanum melongena*) is one of the most common, popular and principal vegetable crop grown in India and other parts of the world. It can be grown in almost all parts of India, except higher altitudes, all the year round. A number of cultivars are grown throughout the country depending upon the yield and consumers preference about the colour, size and shape of the fruits. Phytoplasmas are specialised bacteria which do not have cell wall, obligate parasites found in sieve elements of plants. The term Mycoplasma like Organisms (MLOs) was first used for these micro-organisms due to their morphological and ultrastructural similarity to mycoplasmas. MLOs and mycoplasmas both belong to the *Mollicutes* class, since they are prokaryotes without cell walls[4].Plants infected by phytoplasmas exhibit an array of symptoms include virescence, phyllody, sterility of flowers, witches'-broom appearance, abnormal elongations of internodes resulting in slender shoots, generalized stunting, discolorations of leaves or shoots, leaf curling or cupping, bunchy appearance of growth at the end of the stems, and generalized decline[5].

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Phytoplasmas can be transmitted through grafts; but are not transmitted mechanically by inoculation with phytoplasma containing sap[5]. Twelve phytoplasmas namely Chrysanthemum yellows, *Rehmannia glutinosa*, Ribes in Vinca, Strawberry green petal, Crotalaria saltiana phyllody, Sweet potato little leaf, Vinca coconut phyllody, Plum leptonecrosis, Elm yellows, Potato witches' broom, Brinjal little leaf and Apple proliferation were transmitted from diseased periwinkle to healthy periwinkle by inserting 1.5 cm long sections of diseased stem explants into the stem of the healthy plant directly. All phytoplasmas were successfully transmitted to plants and showed typical disease symptoms 6-8 weeks after transmission. Nested PCR and *SecA* gene sequencing were used to confirm that transmission had occurred[1].

MATERIAL AND METHODS

Little leaf Infected plants of brinjal were used as donor or source plants for the study. The diseased scions from little leaf infected brinjal plants were cut from both sides into a 'V' shaped structure approximately 4 cm. The healthy brinjal rootstocks were first cut straight and after that a perpendicular cut of approximately 4 to 5 cm was made in the center of the stump. The scion was inserted in the cut made in the rootstock and the grafted portion was tied tightly with a high density polythene strip. The grafted portion was covered by polyethylene bags to keep the union moist and the inoculated plants were kept in insect proof net for symptom expression. Observations were recorded on number of plants showing symptoms and time taken for symptom development. Infection of graft-inoculated plants was verified by polymerase chain reaction (PCR). Total genomic DNA from healthy and little leaf infected brinjal plant was extracted by following CTAB (Cetyl Trimethyl Ammonium Bromide) method as described by Kollar *et al*.[2] with some modifications. PCR using P1/P7[6], universal primers, specific to 16S rDNA gene of phytoplasma was performed.

RESULTS

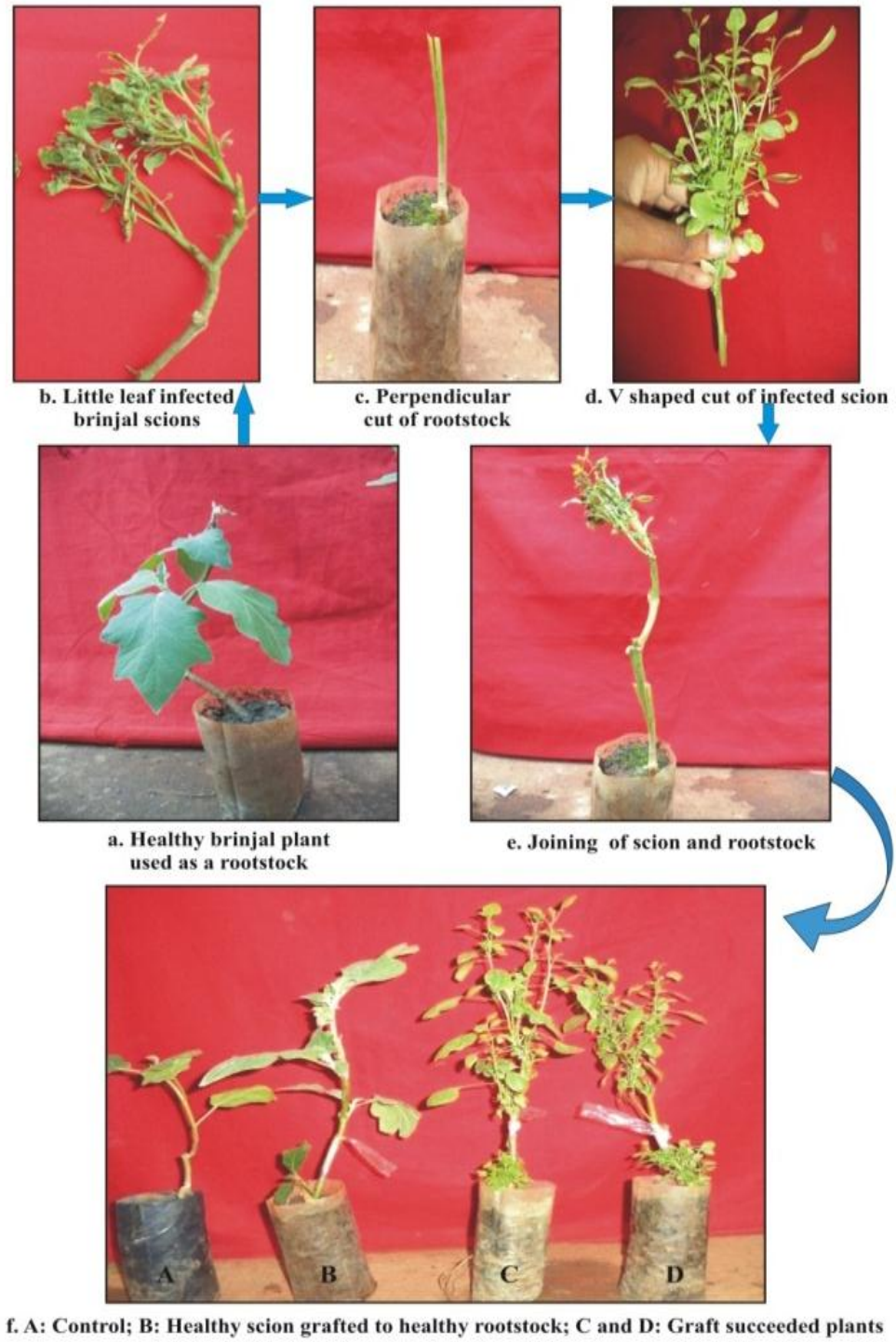
Total twenty brinjal plants were grafted among twenty plants sixteen brinjal plants were graft successful and four plants were not graft successful. Little leaf symptoms was developed in healthy brinjal rootstock plants within 20-25 days after grafting in graft successful plants and there was no little leaf symptoms development in graft failed plants and control plant that was healthy brinjal scion grafted to healthy brinjal rootstock. (Fig.1). Transmission rate of brinjal little leaf phytoplasma was 100 per cent was observed in graft transmission(Table 1).Total genomic DNA was isolated from leaves produced on the brinjal rootstock after graft successful plants and contol plant samples. PCR was done using primers P1/P7 produced 1.8 kb amplicon in only in case of graft successes brinjal plants whereas no such amplicon was produced on the control plants (Fig. 2). This results indicated that the brinjal little leaf phytoplasma was transmitted by wedge grafting from donor little leaf infected brinjal to receptor healthy brinjal plants.

DISCUSSION

The studies on graft transmission indicated that brinjal little leaf phytoplasma was successfully transmitted by wedge grafting from diseased brinjal plants to healthy brinjal plants and needed 20-25 days for expression of symptoms in healthy brinjal rootstock. The phytoplasma transmitted brinjal plants showed typical little leaf disease symptoms like reduced leaf size, virescence, proliferation, phyllody and diminished plant development. This graft transmission fulfilling the Koch's postulates of brinjal little leaf phytoplasma. The results were confirmed by PCR using P1/P7 primer which was positive with the samples from the grafted plants. These results are in accordance with the findings of Lang and Moh-Jih[3], Kawicha *et al*[1].

Table 1: Graft transmission

Type of Grafting	Number of plants			Percent transmission	Time taken for symptom expression (days)
	Total grafted plants	Successful grafted plants	Infected plants		
Wedge grafting	20	16	16	100	20-25



f. A: Control; B: Healthy scion grafted to healthy rootstock; C and D: Graft succeeded plants

Fig. 1: Graft transmission of brinjal little leaf phytoplasma

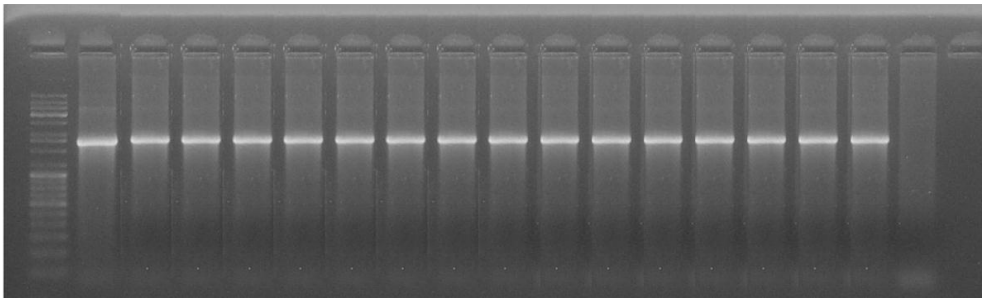


Fig. 2: Polymerase chain reaction confirmation of grafted brinjal plantts

M: Medium range DNA Ladder
Lane 1 to 16: Brinjal little leaf disease infected plants through graft transmission
Lane17: healthy control

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