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Molecular Characterization and Mass Multiplication of Whitefly (*Bemisia tabaci*), a Vector of Ridge Gourd Begomovirus

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ABSTRACT: Ridge gourd is one of the important vegetable crops in India. Ridge gourd cultivation is affected by many biotic and abiotic challenges. Various plant pathogens and insect pests cause significant losses during cultivation. ToLCNDV (Tomato Leaf Curl New Delhi Virus), a begomovirus transmitted by Whitefly (*Bemisia tabaci*) is one of the major limiting factors for Ridge gourd production. The symptoms of virus include chlorosis, mosaic, cupping of leaves, blistering, reduction in internodal lengths. In the present study, Begomovirus in the whiteflies collected from Ridge gourd plants showing typical symptoms of the virus was confirmed using ToLCNDV coat protein specific primers. White flies were collected from the field, species *B. tabaci* was confirmed using mtCOI primers. Whiteflies were mass multiplied in insect proof polyhouses on Brinjal plants for further studies of resistance screening.

Keywords: Ridge gourd, *Bemisia tabaci*, polyhouse, mass multiplication.

INTRODUCTION

Ridge gourd (*Luffa acutangula*), belonging to the cucurbitaceae family, is a very important Indian vegetable crop and it can be grown throughout the year. Ridge gourd vegetable in green stage and leaves with stem are used as vegetables and mainly grown in South and East India.

Ridge gourd is cultivated in an area of about 9920 ha generating a substantial production of about 3.17 lakh tons with a productivity of 31.95 t/ha in India. During the early stage of plant growth, shallow cultivation is preferred. The plants should be provided a suitable support made of bamboo sticks for better creeping. Drip watering is most beneficial in the Ridge gourd cultivation. The summer plantation requires frequent watering at an interval of 3 to 4 days. Normally the rainy season crop does not require any irrigation (Crop production Guide TNAU, 2020).

Ridge gourd crop cultivation is affected with many pest and diseases like Downy Mildew, Powdery Mildew, Bacterial Wilt, Cucumber Mosaic Virus (CMV), Papaya Ring Spot Virus (PRSV), Zucchini Yellow Mosaic Virus (ZYMV), Cucumber Green Mottle Mosaic Virus (CGMMV) and Begomoviruses (Nagendran *et al.*, 2017; Kumari *et al.*, 2021).

Virus diseases that are causing significant yield losses have emerged in the past three decades limiting the production of important vegetable and fiber crops in tropical, subtropical, and temperate regions worldwide. Many of the causal viruses are transmitted by Whiteflies (Hemiptera: Aleyrodidae), mainly by those belonging to the *Bemisia tabaci* cryptic species complex (Reddy *et al.*, 2024). Viruses known to be transmitted by whiteflies include members of the genera *Begomovirus*, *Crinivirus*, *Ipomovirus*, *Torradovirus*, and *Carlavirus* (Navas-Castillo *et al.*, 2011), and two poleroviruses (genus *Polerovirus*) recently reported to be transmitted by *B. tabaci* (Ghosh *et al.*, 2019; Costa *et al.*, 2020).

Tomato leaf curl new Delhi virus (ToLCNDV), a begomovirus transmitted by whitefly (*B. tabaci*) infecting Ridge gourd is an emerging problem in numerous crops and widely distributed in India, Pakistan, Philippines and Thailand (Moriones *et al.*, 2017). Begomovirus produces symptoms such as chlorosis, mosaic, cupping of leaves, blistering and reduction in inter-nodal length by causing yield losses of 30-100% in Ridge gourds in Southern India (Manjunath *et al.*, 2016; Patil *et al.*, 2017). In the present study, we confirmed the whitefly species by molecular methods and further healthy whiteflies were mass multiplied in the insect proof polyhouses to be used in resistance screening of Ridge gourd against Begomovirus.

MATERIALS AND METHOD

A. Whitefly collection and mass multiplication

The pure culture of whiteflies (*B. tabaci*) was collected from brinjal plants from the farmer's field near Doddaballapura, Bengaluru Rural Dist using a field aspirator. Seeds of Brinjal local cultivar (*Solanum melongena* L.) were sown in 4 × 6 cm polybags filled with soil and compost mixture in the ratio of 2:1 inside insect proof polyhouse. Irrigation and fertilizer application was done to maintain healthy seedlings (Patil, 2014).

Collected whiteflies were released onto healthy brinjal plants and allowed to multiply naturally. Temperature of 35±2 °C, 30-50% relative humidity, 8 hours of darkness was maintained inside the polyhouse. Temperature, relative humidity, irrigation schedule was regularly monitored. Dried host plants were replaced regularly with fresh Brinjal seedlings (Rudra Gouda *et al*., 2022).

B. Identification of whitefly species by molecular methods

DNA was extracted from the white flies collected from brinjal plant using CTAB method (Asokan *et al*., 2011). PCR was performed in 50 µl total reaction volume containing 5µl of isolated genomic DNA (50-100 ng/ µl) followed by 2.0 µl of forward primer and 2.0 µl of reverse primer of 20 p moles, 5 µl of 10 mM Tris buffer (pH-8.3), 2.5µl of 2.5 mM MgCl2, 2.0 µl of dNTP mixture (0.25 mM of each dNTP), 30.5µl double distilled water and 1.0 µl (0.5 U concentration) of Taq DNA polymerase (GeNeiTM, Genei laboratories Pvt ltd). PCR amplification was performed in a thermal cycler (Mastercycler X50s; Eppendorf) with the following cycles: 94°C for 2 min as initial denaturation followed by 35 cycles of 94°C for 45 sec, 47°C for 45 sec, 72°C for 45 sec and final extension for 72°C for 10 min. Universal mitochondrial cytochrome oxidase I (CO-I) primer pair (LCO-1490- 5' - GGTCAACAAATC ATAAAGATATTGG -3'; HCO-2198- 5' - TAAACTTCAGGGTGACCAAAAAATCA-3') was used (Hebert *et al.,* 2003). The PCR product was resolved in 1.5% agarose gel, stained with Novel juice (GeneDirex) and documented in a gel documentation system (Vilber, Bio-Print). Sequencing was done using mt-COI forward and reverse primers (Ms. Medauxin, Bengaluru, India). NCBI-BLAST (Basic Local Alignment Search Tool) (http://blast.ncbi.nlm.nih.gov/) tool was used for analyzing the sequence homology. Sequence alignment was done in BioEdit (version 7.0.9.0) (Hall, 1999) and a phylogenetic tree was constructed in MEGA.7.0 software (Kumar *et al*., 1993).

C. Identification of begomovirus in vector whitefly

Whiteflies were collected from symptomatic Ridge gourd plants infected with Begomovirus in a farmer's field. Modified CTAB method was used to isolate genomic DNA from whiteflies (Asokan *et al*., 2011). PCR was carried out by employing the ToLCNDV specific primer ToLCNDV-up GAACTATGGTGAAGCGACCAGCAGA; ToLCNDV-do ACACAGGTCCTTAGGTACCTGG (Alfaro-Fernández *et al.*, 2016) to confirm the presence of virus. Amplified PCR products were sequenced (Ms. Medauxin, Bengaluru, India).

RESULTS AND DISCUSSION

A. Identification of whitefly species by molecular methods

PCR product obtained from the healthy whitefly DNA yielded an expected size of approx size of 650bp (Fig. 1a) using mtCOI primers . Sequencing result of this PCR product confirmed as *B. tabaci* (unpublished data). The sequence blast result showed 100 % identity with other reported *B. tabaci* from India (MW488190) and South Korea (MN532129) isolates. Similar results were observed by various researchers globally (Dinsdale *et al.*, 2010; Himler *et al.*, 2011; Ellango *et al.*, 2015) for whitefly species identification using mtCOI primers.

B. Whitefly collection and mass multiplication

The whitefly population started to multiply with optimum temperature and humidity. During summer, a maximum number of whitefly population were observed on brinjal leaves maintained inside the polyhouse (Fig. 2). Very high and very low temperatures affected multiplication of whiteflies and population were reduced. During cooler nights, temperature was maintained above 25 °C by keeping heaters inside the chambers. Similarly, very high relative humidity and low temperature conditions adversely affected the whitefly population. Abhijit Ghosal (2022) studies on influence of different abiotic factors on whitefly population confirms similar observations.

C. Identification of begomovirus in vector whitefly

The viruliferous whitefly genomic DNA was isolated. Whitefly DNA was amplified with an expected amplicon size of ~ 914bp. ToLCNV CP gene sequence analyzed via BLAST showed an identification of 98.05% with published sequences (KM275616).Sequencing result of this PCR product confirmed as ToLCNDV (unpublished data). Rachmi Putri *et al.* (2023) also conducted similar studies and identification of whitefly collected from the Begomovirus infected leaves was performed. DNA extraction was carried out using lysis and cetyltrimethylammonium bromide (CTAB) buffer, while the presence of *Begomovirus* was detected using polymerase chain reaction.

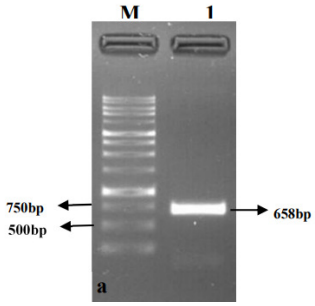

Fig. 1a. PCR amplification of mt COI gene for whitefly identification. M- 1kb ladder; 1-Whitefly (*B. tabaci*).

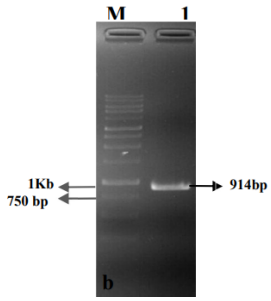

Fig. 1b. PCR amplification of Begomovirus in whitefly using ToLCNDV CP primers. M- 1kb ladder; 1-Whitefly (*B.tabaci*).

Fig. 2. Mass multiplication of whitefly *B. tabaci* on brinjal.

CONCLUSIONS

The findings of whitefly *B. tabaci* molecular characterization signifies the effectiveness of mtCOI gene amplification for precise identification of whitefly species. Confirmation of ToLCNDV, a Begomovirus from the whiteflies collected form virus infected plants confirms involvement of vector *B. tabaci* in virus transmission. Mass multiplication of whiteflies was good with optimum temperature of 35±2°C, 30-50% relative humidity. Whitefly population is reduced during temperatures below 20°C and high humidity. This result concludes the optimum conditions required for whitefly multiplication in any research activities.

FUTURE SCOPE

Screening of different ridge gourd germplasm using vector *B. tabaci* needs to be done to identify the suitable resistance sources to use in resistance breeding and farmer cultivation.

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Conflict of Interest. None.

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