

RESEARCH ARTICLE

Weeds as Reservoirs for Viruses Infecting Brinjal in its Ecosystem

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ABSTRACT

Brinjal is the most important vegetable crop cultivated in both tropical and sub-tropical regions due to its high adaptability and the prevailing demand for edible purpose. Brinjal crop expressing viral disease like symptoms viz., mosaic, severe mosaic, vein banding, blistering, stunting were collected. The weed plants in brinjal ecosystem viz., *Euphorbia* spp. *Parthenium hysterophorus*, *Trianthema portulacastrum*, *Tridax procumbens* and *Eclipta prostrata* were observed with similar symptoms and these symptoms were also collected. The preliminary detection of viruses was carried for Cucumber mosaic virus (CMV) and Tomato leaf curl New Delhi virus (ToLCNDV) antisera resulted in positive reaction for CMV and ToLCNDV infection in *Euphorbia* spp. and *Parthenium hysterophorus* confirmed the infection of CMV and ToLCNDV on these weed plants. The molecular characterization of CMV and ToLCNDV was done and positive amplicon covering coat protein regions of both the viruses were cloned and sequenced. Sequences revealed 98% identity of CMV between the weed species and 97% homology with other vegetable CMV isolates of India. 99% of identity was observed between TN isolates of ToLCNDV and 98% identity with other ToLCNDV Indian isolates. Upon phylogenetic analysis the CMV weed host isolates clustered in single clade, separated from CMV brinjal isolate, while all the three isolates of ToLCNDV claded in single clade. The results exemplified that *Euphorbia* spp. and *Parthenium hysterophorus* act as reservoirs of CMV and ToLCNDV in the brinjal ecosystem during cropping and off-season of the crop in Tamil Nadu.

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INTRODUCTION

Brinjal (*Solanum melongena* L.) is an important *Solanaceae* crop grown in tropical and sub-tropical regions all over the world for its edible fruit and rich nutritive value (Taher et al. 2017). The crop is cultivated in all cropping seasons throughout the year in India, and the total cultivated area in India is around 0.7 million hectares with a production of 12.5 million tonnes and productivity of around 17 tonnes per hectare during (2019-2020) (<https://www.indiastat.com>). India is the second largest producer of brinjal globally, which contributes 24.5% in the global market (<https://en.wikipedia.org/wiki/Eggplant>). The crop profiting is constrained by viruses, and the viruses infecting brinjal in India are Tomato leaf curl New Delhi virus (ToLCNDV)

(Venkataravanappa et al., 2014); Cucumber mosaic virus (CMV) (Kumar et al., 2014); Potyvirus (Bhat et al., 1999) and Eggplant mosaic crinkle virus (EMCV) (Raj et al., 1989). Management of virus disease remains tedious, due to lack of awareness to growers and farmers in the country. The ecology and epidemiology of the viral pathogen have to be studied in detail for the better management of virus diseases in brinjal ecosystem. The present investigation aims to serological and molecular characterization of viruses harboring weed species of brinjal ecosystems of Tamil Nadu.

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MATERIALS AND METHODS

Field observation and symptomatology

A brief field survey was conducted in the brinjal field of TNAU research farm to document viruses infecting brinjal. The infected plants expressed virus-like symptoms viz., mosaic, mosaic mottling, vein banding, chlorosis, puckering and stunting. Apart from brinjal crop weed species viz., *Euphorbia* spp, *Parthenium hysterophorus*, *Trianthema portulacastrum*, *Tridax procumbens* and *Eclipta prostrata* grown in and around brinjal ecosystem were also noticed with virus symptoms like mosaic, yellowing, stunting and curling and symptomatic samples were collected.

Enzyme linked immunosorbent assay

Brinjal and virus-infected weeds samples were subjected to DAC and DAS-ELISA using the polyclonal antibody for CMV obtained from ICAR-NRCB (Indian Council of Agricultural Research-National Research Center for Banana), Trichy, India and ToLCNDV procured from DSMZ, Germany. Experimented with 96 wells polystyrene notch plates with an antigen dilution of 1:1; 1:10 and 1:20 along with healthy samples of brinjal as negative control. About 5 samples of each weed plant and three biological replicates for each dilution were used. Goat anti-rabbit IgG Alkaline phosphatase (1:5000, Sigma Aldrich) was used as conjugate and the optical density (OD) was read at 405 nm (Sunrise™, Tecan groups Ltd, Switzerland).

PCR and RT-PCR

Total genomic DNA and RNA were extracted from symptomatic samples of both brinjal and weed hosts using CTAB and TRIzol® reagent methods using the manufactures direction. First strand of complementary DNA was prepared for RNA samples using Revert Aid First Strand cDNA synthesis Thermo Scientific Kit prior to quantifying the RNA uniformly to 2000ng/μL. Obtained cDNA was subjected to RT-PCR using coat protein gene-specific primer of CMV GKCMV F (5' GAGTTCTCCGCGTCCCGCT) and GK CMV R (5' AACCTAGGAGATGGTTTCA) and DNA samples were analyzed for PCR using universal primer that corresponding to coat protein region of all begomoviruses were used PAL1c1960 (5' ACNGGNAARACNATG TGG GC 3') and PAR1v722 (5' GGNAARATHTGG ATG GA 3').

Cloning, sequencing and computational analysis

Positive amplicons resulting in RT-PCR and PCR analysis were eluted using Sigma elution kit and ligated into p-GEMT easy vector (Promega),

transformed into *E. coli* DH5α strain cells and the positive clones were plasmids extracted and the clones were sequenced at Barcode Bioscience, Bangalore by following Sanger sequencing method. Derived sequences were BLASTn analyzed in the NCBI database to identify viruses infecting both the main crop and the weed species in the brinjal ecosystem. Similar sequences were retrieved from the NCBI database for further analysis. Sequences were aligned using CLUSTAL W, multiple alignments of nucleotide and amino acid sequences were performed with BioEdit sequence alignment editor version 7.0. Phylogenetic tree was constructed using MEGA X software (www.megasoftware.net) with the Neighbor-joining tree method with 1000 bootstrap replication.

RESULTS AND DISCUSSION

Incidence and symptomatology

Brinjal crop exhibited various symptoms like, mosaic, mosaic mottling, vein banding, puckering, blistering and stunting in the diseased plants. Virus-infected weed *Euphorbia* spp. was observed with mosaic, yellowing, mosaic mottling and malformation of entire plant similarly *Parthenium hysterophorus* exhibited leaf curling, malformations and mosaic type of symptoms (Fig.1.). Similar symptoms were documented in weed species infected with CMV, Potyvirus, TSWV and TMV present in and around tobacco fields (Glinka *et al.*, 2021). Correspondingly, sunflower crop infected with TSV were found to be over wintering on *Parthenium* was documented by Bhat and Reddy (2016). Tomato crop in south eastern Brazil were documented to be infected with three begomoviruses that overwinters on six different weed species, grown around tomato ecosystem (Gloria *et al.*, 2008).

Serology

Symptomatic brinjal and weed host plants were analyzed for DAC and DAS-ELISA and the results revealed that the infected samples showed positive reaction for cucumber mosaic virus (CMV) and Tomato leaf curl New Delhi virus (ToLCNDV). Upon DAC-ELISA for CMV the high OD value was observed in brinjal (1.421) followed by *Parthenium hysterophorus* (1.318) and *Euphorbia* spp. (1.267) upon comparison with the positive control (1.356). Similarly, brinjal showed high virus titre for ToLCNDV of (0.962); while *Euphorbia* spp. and *Parthenium hysterophorus* recorded 0.694 and 0.754 OD respectively with the positive control of (0.835). Serological assay experimented for CMV and ToLCNDV revealed that both the viruses showed increasing virus titer according to the dilution used. Other weed species viz., *Trianthema portulacastrum*

(0.064; 0.052), *Tridax procumbens* (0.048; 0.063) and *Eclipta prostrate* (0.0594; 0.048) revealed negative results like healthy control (0.085; 0.076) for both CMV and ToLCNDV (Fig.2.). Abraham *et al.* (2021) reported 12 weeds among 14 weed species in tomato field showed positive titer for Tomato yellow leaf curl virus (TYLCV) in TAS-ELISA.

Molecular characterization

The coat protein gene of CMV infecting brinjal, *Euphorbia* and *Parthenium hysterophorus* had 98, 97 and 98% identity with other 1B subgroup of CMV isolates in India and had 70-73% identity with other II subgroup isolates of India in BLAST search analysis. Between the main host and the weed hosts the identity was found to be 98% while within the two weed species, the identity was found to be more than 99% upon pair-wise alignment. The nucleotide (nt) sequences of all the three virus isolates were deposited in NCBI database [Accession No. TQ226713 (brinjal); TQ226714 (*Euphorbia*) and TQ226715 (*Parthenium hysterophorus*)]. CP gene covers 1130nt converted to 376 amino acid and all the study isolates were positioned with 461R (Arginine or cystine) belonging to mosaic inducing strain of cucumber mosaic virus. Neighbor-joining tree method used for phylogenetic analysis revealed the brinjal and weed isolates viz., *Parthenium* and *Euphorbia* were clustered together and brinjal isolate was clustered with other IB subgroup isolates of other vegetable crops infected with CMV belonging to India (Fig.3.). Akhtar *et al.* (2019) classified the weed infected with CMV as subgroup I through RT-PCR assay which revealed 99% similarity with the main host tomato.

Brinjal, *Euphorbia* and *Parthenium hysterophorus* exhibited 99 and 98% identities with ToLCNDV of vegetables crops of India. Between the three isolates, the identity was 99% upon pair-wise alignment and the study isolates were deposited in NCBI database [Accession No. VR528666 (brinjal); VR528667 (*Euphorbia*) and VR528668 (*Parthenium hysterophorus*)]. In phylogenetic analysis, the study isolates of ToLCNDV were clustered in the same clade with other ToLCNDV isolates of South India (Fig. 4.). Kumar *et al.* (2016) reported that parthenium grown in vegetable eco systems of India was found to be severely infected with many begomoviruses viz., Tomato leaf curl virus (TLCV) and Papaya leaf curl β -satellite (PaLCuB) and Ageratum yellow vein India α -satellite (AYVIA) and they confirmed the harboring nature of the viruses on parthenium, which act as reservoir of inoculum for tomato cropping.

Transmission of viruses through seeds of weed plants plays a crucial role in over wintering the virus inoculum. Parthenium infected with TSV was reported to carry the virus through its pollen and seeds in India (Shanmuga Prema *et al.*, 2020) and Australia (Sharman *et al.*, 2009). CMV and ToLCNDV are seed borne in nature; Akhtar *et al.* (2019) observed that CMV was not transmitted through weed seeds but transmitted with tomato seeds in Pakistan. Congress weed in India also harbors GBNV, CMV and TSV (Vemana *et al.*, 2015) and (Rao *et al.*, 2009). Virus inoculum accumulation in weed plants in the cropping ecosystem creates treats in brinjal profiting and the overall vegetable production in Tamil Nadu.



a



b



c



d



e

Figure 1. Symptomatology in brinjal and weed hosts. (a). mosaic with vein banding in brinjal; (b & c). mosaic and mottling in *Euphorbia* spp. (d & e). Mosaic and leaf curling in *Parthenium hysterophorus*

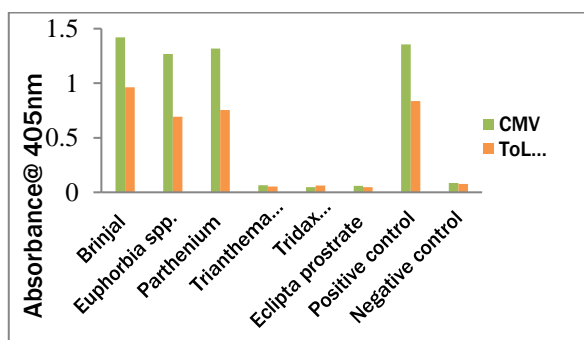


Figure 2. DAC & DAS-ELISA for virus infected weed species in and around brinjal ecosystem

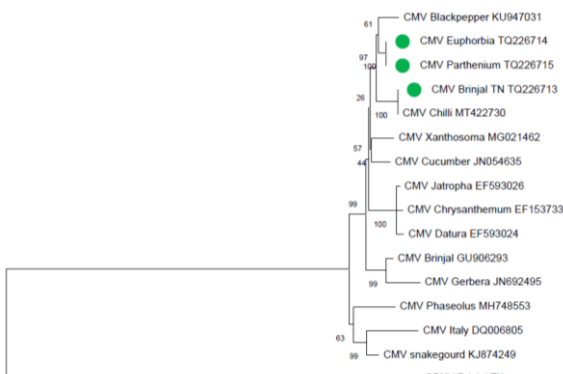


Figure 3. Phylogenetic analysis of CMV isolates of brinjal and weed species with other CMV

isolates. The evolutionary history was inferred from neighbor joining method with 1000 bootstrap replication. Groundnut bud necrosis virus was included as out group

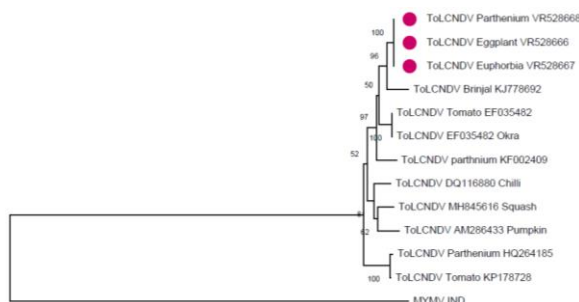


Figure 4. Phylogenetic analysis of ToLCNDV isolates of brinjal and weed species with other ToLCNDV isolates. The evolutionary history was inferred from neighbor joining method 1000 bootstrap replication. Mungbean yellow mosaic virus was included as out group

CONCLUSION

Weeds grown in and around the agricultural land harbors most of the pest and diseases in India during both cropping and off-season of the crop, which plays a vital role in maintaining the life cycle of insects and pathogens. Clean and protected farming has to be practiced in and around the brinjal growing area to break the life cycle of the viral inoculum.

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Ethics statement

The author declares no participation of humans and animal in any of the studies.

Originality and plagiarism

The authors ensured that only totally original works were written and submitted, and that any work and/or words borrowed from others were properly cited

Competing interest

The authors declare that there is no conflict of interest.

Consent for publication

The authors agreed to publish the content.

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail agrikarathi2003@gmail.com.

Authors' contributions

Karthikeyan G designed the project, revised and approved the manuscript for submission; Abirami R drafted the experiments and prepared the manuscript; Manoranjitham S K, and Rajasree V, helped to analyze the data of experiment; Mohankumar S gave proposition to conduct the experiment. All authors reviewed and approved the submission

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