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Emerging Begomovirus ToLCNDV Molecular Detection & Chip Grafting Inoculation Transmission Assay under Natural Conditions in Tomato Plants from District Poonch Azad Kashmir & Pakistan

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Abstract: Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite, emerging Begomovirus species, usually coupled with satellite molecules and affects tomato cultivation in entire Pakistan. Present study was conducted to evaluate and analyze tomato germplasm against ToLCNDV using Chip graft inoculation assay (CGIA) as most accurate and reliable technique. Molecular studies were conducted for confirmation. According to PCR results, viral contamination was confirmed as ToLCNDV was recorded from all tested seedling samples. Further mechanical transmission confirmed that seedlings from ToLCNDV-infected seeds act as inoculum. Based on disease symptoms developed, plants were classified as tolerant, moderately tolerant, moderately susceptible, and susceptible. It is pertinent to mention that the magnitude of the virus impact particularly on hosts different from Solanaceae and *Cucurbitaceae*, and seed transmission and ToLCNDV qualify as a potential candidate of Quarantine Pest. Present work is novel study demonstrating ToLCNDV as a seed-transmissible virus in tomato plants from Rawalakot Azad Kashmir & this research further provides a fictitious starting point for plant pathologists suggesting practical implications regarding management approaches.
Keywords: Begomovirus, Quarantine Pests, Molecular Confirmation

Introduction

Tomato Leaf Curl New Delhi Virus (ToLCNDV) attacks on tomato (*Solanum lycopersicum L.*) at young age and is constant nad serious threat to tomato crop in warmer areas(Prasanna et al., 2015). ToLCNDV causes yield losses up to 90% (Sahu et al., 2010). Epidemics of disease are not only bound to Asia, but the pathogen has altered its geographcal boundaries and now found in even middle east and Iran (Yazdani-Khameneh et al., 2016). Tomato is second most important *solanaceous* crop after potato that is used worldwide. The white fly transmits the ToLCNDV, a bipartite *begomovirus* species (genus *Begomoviral*, family *Geminiviridae*) in a circulative persistent manner (Brown et al., 2012), Bipartite genome (DNA-A and DNA-B) of 2.8 kb in size (Rojas et al., 2005). The DNA A component encodes the coat protein (CP), replication initiation protein (Rep), replication enhancer (REn), and transcriptional activator protein (TrAP), whereas the genes encoding the movement functions are

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located on DNA Bn (Hanley-Bowdoin et al. 1999). The virus (ToLCNDV) is transmitted in circulative, non-propagative, persistent manner and traverse the insect gut, hemolymph, and salivary tissue membranes to reach the salivary glands for transmission (Ghosh et al., 2021).

The very first repoert of ToLCNDV was from india in 1995 affecting solanaceous crops (Padidam et al., 1995) and inn 2012 it entered the geographical boaders of Europe (Ruiz et al., 2015). Host range of virus is diverse and includes chilli pepper, zucchini, cucumber, eggplant and others (Luigi et al., 2019). In 2004 ToLCNDV was reported from Punjab Pakistan in chilli pepper (Hussain et al., 2004).

Tomato is one of most important nutritious crops due to short growing season and high yield value, further tomato contains abundant amount of iron, phosphorus, vitamin B and C (Adenuga et al., 2013). Lycopene is a powerful antioxidant naturally found in tomato that helps prevent the growth of different types of cancer (Khan et al., 2021). Total area of Pakistan under the cultivation of tomato is 150,000-hectare (Jayasuriya et al., 2021). In 2023, production of tomato has reached 5.4 hundred thousand tons, and 5 to 7 grams of tomatoes are consumed per person per day (Khan et al.,2023). The optimal conditions for the proper growth of tomato are 21-24°C in Pakistan (Khokhar and Hri, 2013).

Tomato is produced in almost all the parts of Pakistan. Baluchistan making up 40.3%, followed by Sindh accounting for 25.6%, 19.3% by Punjab, and 14.8% by Khyber Pakhtunkhwa. The production of tomato is reduced due to several diseases, approximately 50% of which are caused by plant viruses. Studies reveal that, about 312 viruses, satellite viruses, and viroid spp have been known to be able to infect tomato crop (Rivarez et al., 2021).Many viral diseases are also associated with this crop that are transmitted by sweet potato white-fly (*Bemisia tabac*i), such as Tomato Yellow Leaf Curl Virus (TYLCV), Tomato Spotted Wilt Virus (TSVW) and Pepino mosaic virus (PepMV), Tomato Leaf Curl New Delhi Virus (ToLCNDV).Whitefly *Bemisia tabacii* is present in tropical and sub-tropical regions as well as in the Mediterranean basin and is carrier of ToLCNDV (Sharma & Prasad, 2017). Mechanical transmission of different isolates is recorded and some varities showed resistance to mechanical transmission of ToLCNDV (Lopez et al., 2015). ToLCNDV oriental melon (OM), isolates were mechanically transmissible in various studies (Chang et al., 2022). According, to research, MP functions as both a virulence and host range determinant.

ToLCNDV spreads systemically and most prevelant in leaves and fruits, the sap transmission ToLCNDV was successful for some selected cucurbitaceous hosts (Sangeetha et al., 2018). Further research has shown that ToLCNDV is also seed transmitted in Zucchini squash plants (Kil et al., 2020). Biological methods such as *Agrobacterium tumefaciens* mediated transfer is also performed but it was too laborious and was not efficient for some genotypes (Al-abdallat et al., 2010), biolistic bombardment is also used but that was also too laborious and expansive and difficult to carry (Lapidot et al., 2007). Transmission of ToLCNDV through chip graft is an efficient and reliable method (Akhtar et al., 2019). In another study by Sangeetha et al., (2018) sap transmission of this begomovirus was successful for some selected *Cucurbitaceae* hosts. The main objective of the present research was to evaluate the success rate of CGIA in tomato plants under natural conditions to further develop the resistant cultivars rapidly.

Materials and Methods

During May-June 2024, leaf samples from infected tomato plants were collected showing different symptoms of ToLCNDV viz; puckering, yellowing, upward and downward curling, mosaic, reduced size and shortening of inter-nodes from District Poonch Rawalakot and CDRI tunnels at NARC

Islamabad. Samples were carried to laboratory for further evaluating the presence of ToLCNDV through molecular technique (PCR).

Chip Grafting Inoculation Assay

To substantiate CGIA, six weeks test plants were inoculated using CGIA (A, B). A slit of 2.5 cm in size was made on 2nd last inter-node of test plant using a sharp scalpel. A succulent branch from the host plant and chip was made from it same as the size of slit on test plant (C), exposing the cambium layer and dipped in distilled water to maintain the moisture level (D). After this, the chip was than inserted in the test plant's slit (E, F), inoculated area was then covered with para-film to avoid the drying or entry of air into it (G) and properly tagged (H), and the host plants were maintained in the natural conditions by following (Akhtar et al., 2019). After one week of insertion, when para-film was expired than the success rate of the union graft was observed visually i.e retained green color, and formation of callus. Leaf samples of inoculated plants were than further tested for the presence of ToLCNDV (Figure 1). Genome Organization of ToLCNDV is diagrammatically explained (Figure 2).

Figure 1: CGIA in Tomato Test Plants

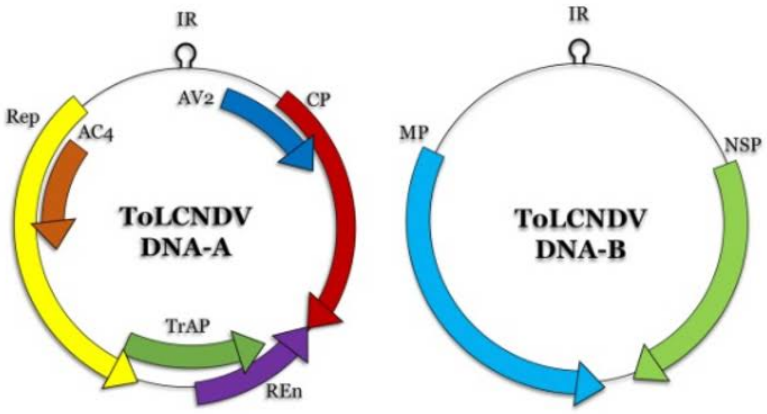

Figure 2: Shows, Genome organization of Tomato leaf curl New Delhi virus (ToLCNDV), ToLCNDV gnomic components DNA-A and DNA-B with arrows showing their respective genes. Genes on DNA-A encode a replication-associated protein (Rep), a replication enhancer protein (REn), a transcriptional activator protein (TrAP), a coat protein (CP), an AV2 protein and an AC4 protein; genes on DNA-B encode a movement protein (MP) and a nuclear shuttle protein (NSP).

Molecular Detection

After 30 DPIs, 4 leaf samples were collected from test plants having Chip inserted in them and DNA was extracted by following the protocol of Doyle and Doyle (1990) and 10µl reaction was carried out using specific primers on already optimized conditions.

Results

Chip Grafting Inoculation Assay

Tomato samples were found positive having both DNA-A and DNA-B. ToLCNDV infected were than maintained and used as the source of chip in Chip Grafting Inoculation Assay. Transmission of ToLCNDV through CGIA was 100% successful, the chip retained its green color and fuse into the rootstock completely and mosaic started appearing after 12-14 DPI. After 18-20 DPI symptoms such as puckering, yellowing, shortening of inter-nodes, upward leaf curling and mottling similar to the symptoms produced by the host plants were observed (Figure 3).

Figure 3: Sample with visible viral symptoms (A) Upward leaf curling (B) Mottling and leaf shortening

Molecular Detection

All the four samples tested were positive and the band size was similar to expected one at 570bp (Figure 4).

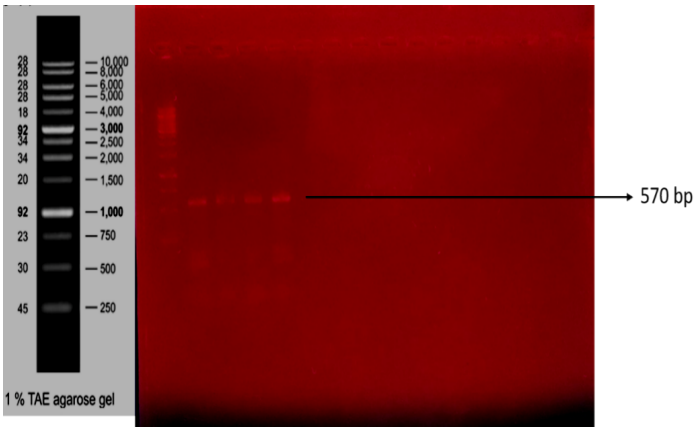

Figure 4: Polymerase Chain Reaction Results of ToLCNDV

Discussion

Viral diseases are most common and destructive threat to the vegetable crops grown all over the world, and their control strategies are more complex as compared to the other pathogens, Viral diseases are transmitted by a vector and the control of vector is also challenging in the field. Various detection methods are used to detect the viral and for molecular characterization of viruses. Hence transmission of viruses is possible through many ways such as a vector-based transmission, through any injury, mechanical transmission, seed transmission and newly introduced Chip grafting inoculation.

As, ToLCNDV is a new emerging virus belongs to begomovirus and is ssDNA bipartite virus having DNA-A and DNA-B, it was reported from India for very first time and found in almost all parts of worlds and affects chili, melons, zucchini, pumpkin and many other vegetable crops but the transmission of virus depends on the host, geographical area and the strain of virus, it affects almost all the members of Cucurbitaceae family and is a major threat to the crops grown. CGIA is successfully reported in Pakistan by (Akhtar et al., 2019), in this method the connection between the cambium layer of a healthy and infected plant is developed, the virus ToLCNDV is phloem limited and it is transmitted by vector whitefly in a circulative persistent manner. So, after inoculation of chip on the healthy plant further evaluations such as, Success rate of chip, Callus formation, symptom development and latent period were done to check the success rate.

Present research emphasizes that all the plants inoculated with the infected chip shows symptoms such as curling of leaves, mottling, mosaic, reduced size that were typical symptoms of ToLCNDV and the further processing such as using of molecular methods such as PCR confirmed that the virus was successfully transmitted to all the inoculated plants. Hence, it is concluded that Chip Grafting Inoculation Assay is an effective and reliable method in the transmission of virus from infected-healthy plants with 100% success rate. Further it is recommended to adopt CGIA as the most reliable, cheap, effective and highly successful method of virus transmission.

Conclusion

ToLCNDV is the most destructive viral disease affecting tomato crop worldwide. Present study emphasizes tomato germplasm against ToLCNDV using Chip graft inoculation assay (CGIA) as most accurate and reliable technique. Further the traditional transgenic techniques hold limited success rate in management of this disease, therefore early molecular detection opens gateway for timely novel management tactics. This is the first reported evidence of ToLCNDV as a seed-transmissible virus in tomato plants from Rawalakot Azad Kashmir & provide a fictitious starting point suggesting practical implications regarding management approaches.

Conflict of Interest: No potential conflict of interest is declared regarding this manuscript.

Author (s) Contribution: Muqadus Zafar performed all experiments; Muqadus Zafar and Hafza Zonash: write original manuscript draft, reviewing and editing. Both the authors have read and agreed to the published version of the manuscript.

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