# Effect of infectious clone of *Tomato leaf curl New Delhi virus* on different tomato cultivars.

**Muhammad Ayyaz Ali[1], Muhammad Azmat Ullah Khan[1], Abdul Qayum Rao[2]**

[1] Department of Biochemistry and Biotechnology, University of Gujrat.

[2]Center for Excellence in Molecular Biology, University of the Punjab, Lahore.

**Abstract-** Improvement of plant biotechnology by transient gene expression owes to the development and refinement of effective tools of transformation. In Pakistan, *Tomato leaf curl New Delhi virus* (ToLCNDV) transmitted by Whitefly is one of the major ascending causes of Tomato leaf curl disease (ToLCD) and limited details are available about tomato natural resistance against ToLCNDV. In this study, agroinfiltration that is a rapid, relatively inexpensive and reliable method was employed for introduction of viral clone in tomato leaves for investigation of inherent resistance against ToLCNDV. PCR reaction confirmed the successful transfer of viral genome transformed in *Agrobacterium tumefaciens* through vacuum infiltration making it a novel study. Out of five locally cultivated cultivars of tomato, Money Maker and Tomato F1 pound were susceptible to ToLCNDV, while remaining three were naturally resistant. Moreover, these susceptible cultivars may also be used for breeding purposes to develop resistance and to increase productivity at commercial level.

***Index terms-*** Agroinfiltration, *Agrobacterium tumefaciens*, Tomato, *Tomato leaf curl New Delhi virus*

## 1. INTRODUCTION

Tomato (*Lycopersicon esculentum L.*) being a vital part of many dishes cooked throughout the world and especially in sub-continent [1]. Its production has been low in Pakistan due to a number of biotic and abiotic factors like insects, fungi, bacteria, viruses, drought, salinity etc. Throughout the world nearly 25 types of viruses infecting tomato have been described so far [2]. *Geminiviridae* family has been classified into 9 genera. This classification depends on genome structure, type of vector and range of hosts. This family has approximately 300 species having circular ssDNA genomes [3].

Tomato leaf curl disease (ToLCD), in the subcontinent, is considered to be convicted by 7 monopartite viruses containing only one genomic element and 2 bipartite viruses consisting of two genomic elements viz., DNA-A and DNA-B. The bipartite viruses *Tomato leaf curl New Delhi virus* and *Tomato leaf curl Gujarat virus* are prevalent in this region [4].

The *tomato leaf curl New Delhi virus* (ToLCNDV) is a big hurdle to tomato production in Pakistan and some parts of India [5]. It infects a huge number of plants of families *Cucurbitaceae*, *Malvaceae* and *Solanaceae*. Major symptoms are upward or downward curling of leaves, stunting, thickening of veins and also leaves with reduced size [5]. Being the member of family *Geminiviridae*, it infects only dicotyledonous plants hence may not have satellite DNAs commonly associated with it [6]. However naturally satellite DNAs particularly betasatellites may coexist with its infection that can regulate its host range and pathogenicity [7, 8]. Its transmission is naturally performed by the vector, whitefly (*Bemisia tabaci*) with the aid of a circulative and unrelenting process. Bipartite begomoviruses contain six open reading frames (AC1, AC2, AC3, AC4, AV1 and AV2) on DNA-A component encoding six proteins for virus replication, transcription, pathogenicity and encapsidation. Two open reading frames named as BC1 and BV1 on B-component encode two proteins for the movement and gene products of the virus. It's both DNA members (A and B) are of same size of ca. 2.8 kb [9, 10]. Both components have an identical sequence called as common region that is

responsible of replication of DNA-B with the help of a protein called replication initiator protein whose gene is present on DNA-A [9].

Many of the control strategies like weed extermination and cultural practices proved to be impotent. Insecticides may prove to be fruitful to some extent but its consistent use can develop resistance in its vector *B. tabaci* and also environmental hazards. The epidemiological factors like viral high evolutionary rate, broad host range and vector's migratory behavior have made it very arduous to evolve adequate control strategy [11]. That is why breeding programs have been emphasizing on genetic improvement of tomato in the growing areas [12, 13]. No resistance has been reported in any tomato cultivar in Pakistan against ToLCNDV to date. Hence an efficient essay work is required to evaluate different tomato cultivars resistance against ToLCNDV.

The agroinfiltration protocol is an effective strategy for delivery of viral DNA clone transformed in *A. tumefaciens* into the plant for infection purposes.*A. tumefaciens* with cloned viral DNA does not mimic the transmission by whitefly, as its farming is very laborious and also very controlled conditions are required for infection by *B. tabaci* [14, 15].

This research work deals with vacuum agroinfiltration of ToLCNDV in the leaves of five of locally cultivated tomato cultivars in Pakistan. As a result of this research work, we have examined natural resistance of different tomato cultivars in a controlled optimized growth laboratory environment.

## 2 MATERIALS AND METHODS

### 2.1 Growing tomato plant cultivars

Five locally cultivated tomato cultivars Remus (V1), Anmol (V2), Tomato Romaking (V3), Money Maker (V4), Tomato F1 pound (V5) seeds were obtained from local seed market of Gujrat and sowed in small pots in controlled laboratory conditions i.e., temperature 25± 2°C, in 25 $\mu Mm^{-2}s^{-}$ of light and 70 % of humidity. Two weeks old seedlings were shifted to fresh pots for the sake of replantation and were kept at same temperature but in light of 75 $\mu Mm^{-2}s^{-}$ for 8 h and 16 h dark period. This work was performed in the Biotechnology laboratory of Department of Biochemistry and Biotechnology, University of Gujrat, Pakistan.

### 2.2 Infectious clone transformation and agroinfiltration

ToLCNDV infectious clone in pCAMBIA1302 was transformed into *Agrobacterium tumefaciens* LBA14044 by heat shock method [16]. L.B agar plates supplemented with 50 µg/mL each of Kanamycin and Rifampicin were used to obtain positive transformants. Colonies of transformants were transferred to LB broth containing 50 µg/mL each of Kanamycin and Rifampicin and incubated at 28° C overnight at 150 rpm in a shaking incubator. This culture was used as an inoculum for fresh culture and after 4 h *A. tumefaciens* broth was pelleted down at 4000 rpm for 10 min. Pellets were resuspended in MES infiltration buffer (10mM MES pH 5.7, 10 mM $MgCl_2$, 150 µM acetosyringone) with $OD_{600}$~0.5. After 2 h incubation at room temperature, recombinant culture containing infectious clone was injected in the lower epidermis of 4-5 weeks old tomato leaves through vacuum infiltration with the help of a 1 mL needleless syringe [17]. Experiments were performed in triplicate with a wild culture of *A. tumefaciens* as control.

### 2.3 Screening plants for infection

ToLCNDV infection was confirmed by observing physiological disease symptoms of infiltrated plant leaves after 7 days of post infiltration (dpi). For virus presence confirmation total DNA was extracted from symptomatic and non-symptomatic leaves using Cetyltrimethyl ammonium bromide (CTAB) method [18] and PCR with 58° C annealing temperature was performed for DNA-B genome. PCR product was confirmed by running 1% agarose gel electrophoresis.

# 3 RESULTS

## 3.1 The physiological appearance of leaves

Tomato cultivars V1, V2 and V3 showed no symptomatic signs of ToLCD at all, proving that these cultivars are naturally resistant to ToLCNDV. Money Maker (V4) and Tomato F1 pound (V5) showed clear symptoms like reduced leaf size, curling of leaflets (**Figure 1**. A) and deformed leaflets, stunted growth (**Figure 1**. B) respectively, hence were susceptible to ToLCNDV.

## 3.2 Confirmation of infection through PCR

To confirm presence of viral genome in plant PCR of conserved region was performed using forward 5'--ACGCTGATTTAGCTCCCTGA--3' and reverse 5'--CCCAATTGCAAACCCTAGAA--3' primers of DNA-B genome of all five samples of total DNA extracted through CTAB method. PCR products (**Figure 2**) were obtained in all samples confirming the successful infiltration of viral genome into the tomato leaves irrespective of appearance of disease symptoms.

# 4 DISCUSSION

Certain approaches are necessary to study viral diseases of plants in a controlled environment for better understanding of their mode of action and to enquire resistance in host [19]. In the past, different methods of inoculation (single leaflet grafting, side-veneer, chip grafting and top wedge) relied on obtaining heirs from naturally infected plants showing ToLCD symptoms that minimize the experimental replicates. Also waiting for natural infection and search for infected symptomatic plants make these methods very strenuous, incompetent and exorbitant when used for a large-scale screening method. Hence the current study has tried to pave the path for a simple, cost effective and fast agroinfiltration method to overcome all the back draws of above methods.

The agroinfiltration assay method has many advantages over previous graft inoculation methods. It can be performed on large scale due to culturing of transformed *A. tumefaciens* containing infectious clone of ToLCNDV as a large number of chips are required in case of single leaflet, top wedge, side-veneer and chip graft inoculation techniques. In addition, this method doesn't need a chip or heir from an infected plant, hence it reducing the time for screening and searching the infected plants in the field. Agroinfiltration has been performed in five different locally cultivated tomato cultivars in Pakistan. Its results showed that it is a triumphant method and gave 100 % success ratio.

During this study, three tomato cultivars V1, V2 and V3 showed inherent natural resistance against ToLCD and the remaining two cultivars money maker V4 and tomato F1 pound V5 gave the symptomatic signs of this disease, proving them susceptible to the virus. The appearance of the disease in two cultivars gives insight into searching for resistance strategies against ToLCNDV. The process of infection may not depend on a single factor. Experimental conditions like inoculation at early stages and high infiltration pressure, were favorable for effective transmission of virus in the plant leaf tissues. Through these findings, in little time infection can be introduced easily in tomato in a controlled laboratory for research purposes.

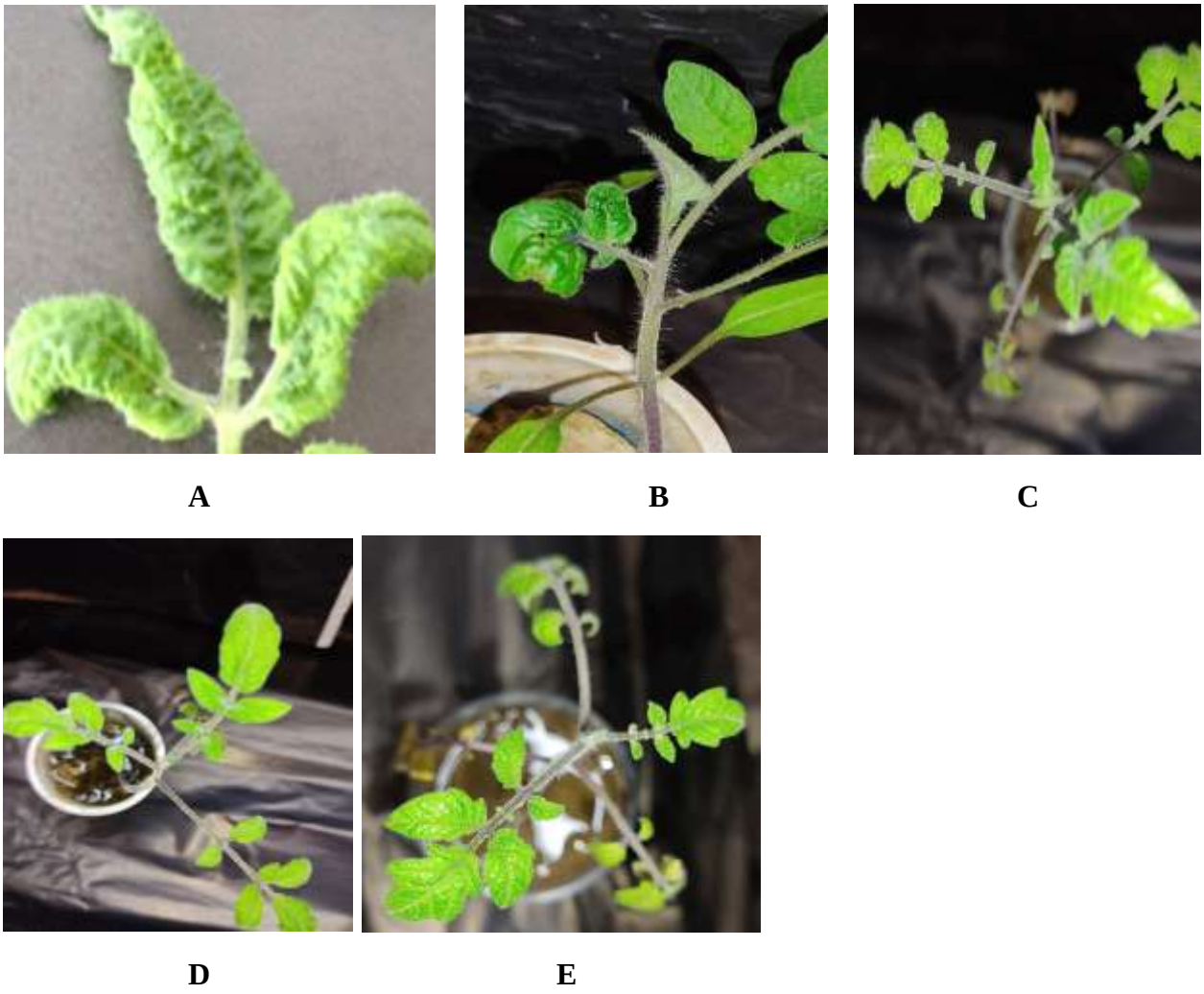


**Figure 1**. After 7 days of post infiltration (dpi) symptoms observed in plants **A**. downward curling in V4 **B**. mosaic and vein clearing in V5, **C-E**. V1-V3 with no symptoms like uninoculated healthy plants

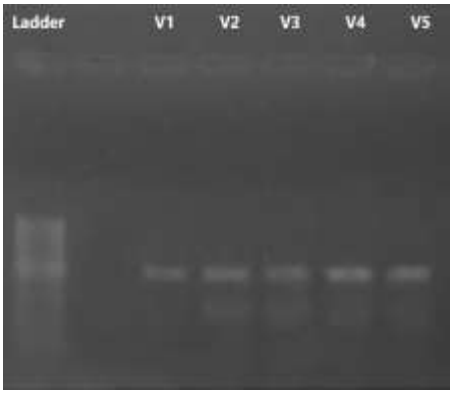


**Figure 2.** PCR result of 5 cultivars with a ladder 100 bp in well-1, wells 2-4 contain V1 -V3, wells 5,6 contain V4 and V5

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AUTHORS

**First Author** – Muhammad Ayyaz Ali, M.Phil, Department of Biochemistry and Biotechnology, University of Gujrat,

**Second Author** – Muhammad Azmat Ullah Khan, Ph.D, Department of Biochemistry and Biotechnology, University of Gujrat,

**Third Author** – Abdul Qayum Rao, Ph.D, Center for Excellence in Molecular Biology, University of the Punjab, Lahore.

**Correspondence Author** – Muhammad Azmat Ullah Khan,