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Development of an efficient in vitro-inoculation method for *Tomato leaf curl virus* on *Lycopersicum esculentum*

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Abstract: ***Tomato Leaf Curl viral Disease (TLCD) is a widely studied viral infection caused by the Geminivirus, TLCV, leading to enormous crop loss across the world. There are more than 21 varieties of the virus reported from India. However, each viral variety is specific in its infection pattern leading to variation in the survivability and symptoms development on the plant. To enable the invitro study of the virus it is therefore, mandatory to standardize the methods for mechanical infection of the virus. The current study therefore focuses on understanding the effect of age of plant, load of viral inoculums, pattern of inoculation including percentage leaves infected and effect of addition of charcoal during inoculation, on the symptoms development on the plant. From the above experiments an efficient in vitro-inoculation method for Tomato leaf curl virus on Lycopersicum esculentum was developed.***

Keywords:TLCD,TLCV, Geminivirus.

Introduction:

Tomato (*Lycopersicon esculentum* Mill.) is an important and most widely grown vegetable crop in India. It is considered to be fairly high in vitamins A and C. Tomatoleaf curl virus (TLCV), a geminivirus(Geminiviridae) is the most important anddestructive viral pathogen in many parts of India (Saikia and Muniyappa, 1989; Harrison *et al.*, 1991).Tomato leaf curl disease(TLCD) is the most devasting disease affecting tomato plants characterized by severe leaf curling, shrinking of tomato leaves, and stunted plant growth. So far, 21 tomato leaf curl viruses have been reported on tomato in India. The pattern of infection for each variety is differing from other viruses.Gujarat has one of the highest yields in tomato production at 22197kg/hector.Recently, TLCVhas become the prime limiting factor in tomato production in Gujarat. As the disease causes heavy yield loss it is important to study the infection pattern and suitable management practices.The pattern of infection, percentage of viral sample inoculum and age of plants at the time of infection affect the stage of symptoms development after infection, pattern of infection and survivability of tomato plants.The data was analyzed statistically and results were interpreted.

Materials & Methods:

1. **Extraction of virus:**Maintenance of viral cultures from infected plant leaves wasdone by three methods.

   A.Maintenance by direct freezing of leaves: Plant leaves showing TLCV symptoms were preserved at low temperature, 4°C temperatureand checked for the viability of the virus.

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B.Maintenance by dehydrated $CaCl_2$: Leaves with typical TLCV symptomswere collected and stored over dehydrated $CaCl_2$. Plant leaves were stored at 4°C temperature and were checked for the viability of the virus.

C.Maintenance by collection of sap: TLCV inoculums was prepared by grinding 1gm symptomatic leaves of tomato plants in 0.1M Potassium Phosphate Buffer, pH 7.0 containing 0.15% Sodium Sulfite. Saps were maintained at 4°C temperature and were checked for the viability of the virus.

2. **Inoculation of plants**

The soil was sterilized and mixed with sand in 1:1 ratio and distributed equally in pots The seeds were then planted. Pots were kept in full sunlight. Plants were watered after every 2 days. Experimental plants infected with TLCV by rubbing with the sap inoculums using a cotton wrapped stick and were observed for symptoms development. Seeds were also planted to be grown as control plants.

A. *Effect of inoculation of plants using charcoal on leaves / in sap:*
Two sets of experimental plants were maintained. In one the test plants were rubbed with charcoal powder followed by applying sap on the leaves. Whereas in the other set test plants were inoculated by rubbing with sap of infected plants containing charcoal powder. Control plants were not inoculated.

B. *Effect of percentage of plant leaves infected:*
To check the effect of the extent of inoculation on the infection pattern. 10, 50 and 100% leaves were inoculated with the viral samples and observed for symptoms development and survivability of plants. The leaves were infected with sap of infected plants. Control plants were notinoculated.

C. *Effect of pattern of infection:*
For this study two sets of experimental plants were maintained.Newly formed young upper leaves of experimental plants were infected with sap collected from infected plants, in one set. Whereas inanother experiment older lower leaves of the experimental plants were infected with the sap. Control plants were not inoculated. The plants were checked for symptoms development and survivability.

D. *Effect of plant age at the time of inoculation:*
This study was done to check the susceptibility of tomato plants TLCV at the different stages of growth. Tomato plants of different age viz. from 15 days, 20 days, 25 days, 30 days and 35 days were inoculated with sap of infected plants without inoculating the control plants. Plants were monitored for their survivability.

Results:

1. **Extraction of virus:**Samples fromall the three maintenance methods were viable and helped in development of symptoms but among all methods maintenance by collection of sap showed maximum viability viz.upto 6 months.
2. **Inoculation of plants:**

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A. *Effect of inoculation of plants using charcoal on leaves / in sap:*

As it's seen in the *figure (1)*rubbing of charcoal on leaves caused blackening in leaves followed by early death of plants.

(a) *(b)*

Figure 1: Effect of addition of charcoal during inoculation of TLCV; (a) Rubbing charcoal on leaves; (b) Addition of charcoal in the sap

In *figure (2)* it is clearly observed that addition of charcoal in sap and using it as inoculum on leaves showed superior results. Symptoms were clearly observed.

B. *Effect of percentage of plant leaves infected:*

No.	% of Leaves Infected	Symptoms Development (Days after infection)	Survival of Plants after infection
1	100%	2 days	-
2	50%	4 days	-
3	10%	12 days	+

Table 1: Effect of percentage of plant leaves infected on symptom development and survivability of plants

Early death of plants was observed when 50-100% plant leaves were infected. Although symptoms were observed within 2 days but the infected plants were not able to survive for longer periods of time. Whereas, in plants where only 10% of plant leaves were inoculated, although symptoms developed 12 days after inoculation and the plants survived (Table 1).

C. *Effect of pattern of Infection:*

Newly formed young leaves inoculated with sap caused early development of symptomshowever it wasfollowed by early death of plants. Whereas, older leavesinoculated with sap helped in observation of symptoms and survival of plants.

D. *Effect of plant age at the time of inoculation:*

No.	*Age of plant at the time of infection*	*Survival*	*Developing time for symptoms*
1	15 days	+	12 days
2	20 days	+	14 days

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3	25 days	+	14 days
4	30 days	+	16 days
5	35 days	+	17 days

Table 2: Effect of plant age at the time of inoculationon symptom development and survivability of plants

From the above table it wasconcluded that plant age at the time of inoculation didn't affect the plant survivability.

Conclusion:

In the current study influence of parameters such as mode of inoculation, size of inoculum, site and extent of inoculation, plant age at the time of inoculation on infection pattern and survival of *Lycopersicum esculentum* were analyzed. It was observed inoculating 10% of the plant leaves, 15days after sowingby rubbing with a cotton swab on older, lower leaves,with30 µg of sap containing charcoal gave the best results.

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