

Screening for natural occurrence of tomato leaf curl virus (TLCV) in devipatan mandal of Tarai region of Uttar Pradesh

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SUMMARY

Investigations were made to identify the leaf curl virus associated with tomato under natural conditions. The predominant symptoms evoked by isolate TLC-I were vein banding, yellowing, slight curling, straight branching and stunting while those of isolate TLC-II were vein thickening, puckering, excessive leaf rolling. None of the isolates were sap or seed transmissible. Aphid species also failed to transmit the isolate. But both the isolates were successfully transmitted by grafting and whitefly (*Bemisia tabaci*, Genn.). Both the isolates reacted positively in triple antibody sandwich ELISA (TAS-ELISA) test with the monoclonal antibodies against Indian tomato leaf curl virus. Electron microscopic studies revealed that both the isolates consisted of geminate particles. In immunosorbent electron microscopy (ISEM), both the isolates exhibited enhanced trapping of virion with antiserum against Indian cassava mosaic virus (ICMV), a geminivirus. Both the isolates were found to be tomato leaf curl virus.

Key words :

Tomato, Tomato leaf curl virus (TLCV).

Tomato (*Lycopersicon esculentum*, Mill.), family solanaceae is one of the most popular and extensively grown fruit vegetable crop in the world. Among the different diseases of tomato, the leaf curl is most widespread and destructive disease, as sometimes it leads to cent per cent crop loss (Butter and Rataul, 1981, Saikia and Muniyappa, 1989, Ansari and Tewari, 2004). The tomato leaf curl virus (TLCV) is transmitted by whitefly (*Bemisia tabaci* Genn.) (Vasudeva and Samaraj, 1948). In Uttar Pradesh this disease is widely prevalent and estimated to cause 27-40% loss (Ansari *et al.*, 2005). The predominant symptoms of the disease are vein clearing, vein banding, reduction in leaf size, stunting, marginal and severe curling of leaves. Puckering of leaf is also common. Since the disease causes serious loss, an investigation was made to identify the association of viruses causing leaf curl disease in tarai region of Uttar Pradesh.

MATERIALS AND METHODS

Surveys of tomato fields were conducted in different tehsils of four districts constituting the Devipatan mandal of tarai region of Uttar Pradesh, to record the distribution and incidence of leaf curl disease. Among different varieties of tomato which are most commonly sown in this area, three varieties namely Pusa Ruby, Punjab Chuhara and Pusa Early Dwarf were

taken for the study. The diseased and healthy plant counts were taken from 2 x 2m². area of five random places in each of five fields in every tehsil and per cent disease incidence (PDI) was calculated by following formula:

$$PDI = \frac{P_1 \times 100}{P_2}$$

where, P₁ = Number of infected plants

P₂ = Total number of plants

At the same time based on symptoms, 14 isolates were designated as T₁ to T₁₄ for each variety of tomato chosen for study. These isolates were transmitted to healthy seedlings of tomato by whitefly inoculation under insect proof glasshouse conditions and were maintained separately in isolation chambers. The prominent symptoms induced by 14 isolates of three different varieties were recorded (Table 1, Table 2, Table 3 and Plate 1).

On the basis of their reaction on certain biological indicator plants (Table 1, Table 2 and Table 3), these isolates were further categorised into two groups (Table 4). A representative isolate from each group was selected and redesignated as TLC-I and TLC-II for further detailed investigations on symptomatology, transmission, particle morphology and serology.

Identity of the virus was finally established by triple antibody sandwich ELISA (TAS-

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Table 1 : For Pusa Ruby variety of tomato

Locality(Tehsils)	PDI (%)	Isolate	<i>D. stramonium</i>	<i>D. metel</i>	<i>N. tabacum</i>	<i>N. rustica</i>	<i>C. album</i>	<i>C. murale</i>	Prominent symptoms
Dist-Bahraich									
Bahraich Proper	27.6	T ₁	+	+	+	-	-	-	GVB,LY,SC,SB
Nanpara	35	T ₂	+	+	+	-	-	-	CL,LY,SB
Kaiserganj	31.8	T ₃	+	-	-	+	-	-	SLR,VT
Mahsi	37.5	T ₄	+	+	+	-	-	-	GVB,LY,SC
Dist-Balrampur									
Balrampur Proper	34	T ₅	+	-	-	+	-	-	SLR,PV
Tulsipur	39	T ₆	+	+	+	-	-	-	GVB,SC,SB
Utraula	30.4	T ₇	+	+	+	-	-	-	CL,LY,SB
Dist-Gonda									
Gonda Sadar	29	T ₈	+	-	-	+	-	-	SLR,PV,VT
Tarabganj	32	T ₉	+	-	-	+	-	-	ELR,VT
Mankapur	36.2	T ₁₀	+	+	+	-	-	-	CL,LY,SB
Karnailganj	34	T ₁₁	+	-	-	+	-	-	SLR,PL,VT
Dist-Shravasti									
Bhing Proper	32.5	T ₁₂	+	-	-	+	-	-	SLR,PV
Ikauna	30.8	T ₁₃	+	+	+	-	-	-	LY,SC,SB
Jamunha	37	T ₁₄	+	+	+	-	-	-	GVB,LY,SC

Table 2 : For Punjab Chuhara variety of tomato

Locality (Tehsils)	PDI (%)	Isolate	<i>D. stramonium</i>	<i>D. metel</i>	<i>N. tabacum</i>	<i>N. rustica</i>	<i>C. album</i>	<i>C. murale</i>	Prominent symptoms
Dist-Bahraich									
Bahraich Proper	27	T ₁₅	+	-	-	+	-	-	SLR,PV,VT
Nanpara	29.5	T ₁₆	+	+	+	-	-	-	GVB,LY,SC,SB
Kaiserganj	24	T ₁₇	+	+	+	-	-	-	CL,LY,SB
Mahsi	30	T ₁₈	+	-	-	+	-	-	ELR,VT
Dist-Balrampur									
Balrampur Proper	26.4	T ₁₉	+	-	-	+	-	-	SLR,PV
Tulsipur	31	T ₂₀	+	+	+	-	-	-	CL,LY,SC
Utraula	28.5	T ₂₁	+	-	-	+	-	-	SLR,PV
Dist-Gonda									
Gonda Sadar	25	T ₂₂	+	-	-	+	-	-	SLR,PV
Tarabganj	30	T ₂₃	+	+	+	-	-	-	CL,LY,SB
Mankapur	29.2	T ₂₄	+	+	+	-	-	-	CL,SB
Karnailganj	27	T ₂₅	+	-	-	+	-	-	ELR,VT
Dist-Shravasti									
Bhing Proper	28	T ₂₆	+	-	-	+	-	-	SLR,VT
Ikauna	30.5	T ₂₇	+	+	+	-	-	-	GVB,LY,SC
Jamunha	25	T ₂₈	+	+	+	-	-	-	LY,SC,CL

ELISA) using monoclonal antibodies against tomato leaf curl virus. Immunosorbent electron microscopic studies were conducted by using antisera to Indian cassava mosaic virus (ICMV). Only trapping was studied employing the procedure described by Derrick (1973).

Percentage disease incidence and differential reaction of virus isolates of varieties of tomato namely

Pusa Ruby, Punjab Chuhara and Pusa Early Dwarf on various alternate hosts in different tehsils of four districts of Devipatan mandal of tarai region of Uttar Pradesh, INDIA.

RESULTS AND DISCUSSION

The average per cent disease incidence was highest

Table 3 : For Pusa Early dwarf variety of tomato

Locality (Tehsils)	PDI (%)	Isolate	<i>D. stramonium</i>	<i>D. metel</i>	<i>N. tabacum</i>	<i>N. rustica</i>	<i>C. album</i>	<i>C. murale</i>	Prominent symptoms
Dist-Bahraich									
Bahraich Proper	13	T ₂₉	+	+	+	-	-	-	CL,SC,SB
Nanpara	18.5	T ₃₀	+	-	-	+	-	-	SLR,PV,VT
Kaiserganj	9.5	T ₃₁	+	-	-	+	-	-	ELR,VT
Mahsi	15	T ₃₂	+	+	+	-	-	-	GVB,SC,SB
Dist-Balrampur									
Balrampur Proper	17.4	T ₃₃	+	-	-	+	-	-	SLR,PV
Tulsipur	21	T ₃₄	+	+	+	-	-	-	CL,LY,SB
Utraula	19.5	T ₃₅	+	-	-	+	-	-	ELR,VT
Dist-Gonda									
Gonda Sadar	10.8	T ₃₆	+	-	-	+	-	-	SLR,,VT
Tarabganj	18	T ₃₇	+	+	+	-	-	-	GVB,LY,SC,SB
Mankapur	20.3	T ₃₈	+	+	+	-	-	-	CL,LY,SC
Karnailganj	16	T ₃₉	+	-	-	+	-	-	SLR,PV
Dist-Shravasti									
Bhinga Proper	15	T ₄₀	+	+	+	-	-	-	CL,LY,SB
Ikauna	17.5	T ₄₁	+	-	-	+	-	-	SLR,PL,VT
Jamunha	20	T ₄₂	+	+	+	-	-	-	LY,SC,SB

GVB=green vein banding, LY=leaf yellowing, SC=slight curling, SB=straight branches, SLR=slight leaf rolling, ELR=excessive leaf rolling, PV=purple veins, VT=vein thickening, CL=curling of leaves, PL=puckering of leaves

(39%) in Tulsipur tehsil of district Balrampur and lowest (9.5%) in Kaiserganj tehsil of district Bahraich in Devipatan mandal of Uttar Pradesh. Out of three varieties of tomato undertaken for study, Pusa Ruby was found to be most susceptible and Pusa Early Dwarf was most resistant. The disease incidence is also higher in the fields with monocrop of tomatoes than in fields where tomatoes were intermixed with other crops like egg plant, cucumber or corn plants (Al-Musan).

Based on the reactions on biological indicator plants, 14 virus isolates were categorised into two groups (Table 4). A representative isolate from each group was re-designated as TLC-I and TLC-II. None of the isolate were sap or seed transmissible. All aphid species tested failed to transmit both the isolates. The virus isolates were successfully transmitted through grafting and by using whitefly (*Bemisia tabaci*, Genn.).

Symptomatically, isolate TLC-I resembled with tomato leaf curl virus that was earlier reported from Bangalore (Saikia and Muniyappa, 1989). It causes reduction in leaf size, leaf curling and stunting of plants. This isolate also resembled in symptomatology with tomato yellow leaf curl virus reported by Louro *et al.* (1996). It also resembled with tomato yellow leaf curl virus reported by Mazyad *et al.* (1979) and it causes reduction and stunting of plants and abscission of flowers. Likewise the isolate TLC-II resembled in symptoms to leaf curl disease of tomato reported by Vasudeva and Somaraj (1948) from New Delhi, India.

In immunosorbent electron microscopy (ISEM), both isolate exhibited enhanced trapping of the virions with antiserum against Indian cassava mosaic virus (ICMV). In TAS-ELISA, both the isolates reacted positively with the monoclonal antibodies against Indian tomato leaf curl

Table 4 : Grouping of isolates on the basis of their reaction on indicator plants

Re-designation of representative isolate	Groups of isolates	Representative isolate	Differentiating symptoms	Incubation period
TLC-I	T ₁ , T ₂ , T ₄ , T ₆ , T ₇ , T ₁₀ , T ₁₃ , T ₁₄ , T ₁₆ , T ₁₇ , T ₂₀ , T ₂₃ , T ₂₄ , T ₂₇ , T ₂₈ , T ₂₉ , T ₃₂ , T ₃₄ , T ₃₇ , T ₃₈ , T ₄₀ , T ₄₂	T ₁	Green vein banding, yellowing, slight curling, slight branches	23-26
TLC-II	T ₃ , T ₅ , T ₈ , T ₉ , T ₁₁ , T ₁₂ , T ₁₅ , T ₁₈ , T ₁₉ , T ₂₁ , T ₂₂ , T ₂₅ , T ₂₆ , T ₃₀ , T ₃₁ , T ₃₃ , T ₃₅ , T ₃₆ , T ₃₉ , T ₄₁	T ₃	Vein thickening, purple veins, excessive leaf rolling, puckering of leaves	19-23



Plate 1 : Tomato fields surveyed showing different sites in Devipatan mandal of Uttar Pradesh

virus.

Hence based on the different identification parameters including symptomatology, particle morphology and serological relationship, the isolates were identified as belonging to subgroup III of the genus Archaeogeminivirus, sub-family Betageminivirinae and family Geminiviridae and found to be tomato leaf curl virus (TLCV).

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