

The Anatomic responses of *Lycopersicon esculentum* Mill. due to the Tomato Leaf Curl Virus (TLCV)

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Abstract

An anatomical study of tomato plants infected by tomato leaf curl virus was conducted to elucidate the mode of infection of the causal virus. One to two months after whitefly transmission, the severe symptoms appear with thickening of the veins, curling of leaf and stunting of plant. Typically reorganization of leaf tissue consisted in replacement of the spongy parenchyma by a palisade parenchyma. Palisade parenchyma tissues were compact in comparison to healthy. Abnormal cambial activity was observed in conducting tissue. Weaker sclerenchyma rings were narrow and these were fewer narrow xylem vessels. Phloem necrosis was observed frequently in virus infected stem. Bronzing & discoloration sieve elements in phloem were also found in infected stem. The cortical parenchyma was wider and formation of mechanical and conducting tissues was reduced. In root of infected plant secondary thickening was less in comparison to healthy. Xylem vessels were narrow and with scanty phloem in diseased root. The no. of stomata was also reduced in infected leaves.

Keywords: *Anatomical changes, Tomato, TLCV, Stomatal index*

Introduction

Tomato leaf curl is a serious disease of tomato (*Lycopersicon esculentum* Mill.) that has been spreading in intensive tomato production area of eastern U.P. This disease can cause 50-90% yield loss (Sastry and Singh, 1973; Saikia and Muniyappa, 1986, 1989). However, the incidence occasionally reaches up to 100%. TLCV infected plants show a variety of symptoms including leaf curling, vein clearing yellowing and stunting etc. Tomato leaf curl disease is caused by Geminivirus (Genus-Begmovirus) and is transmitted by *Bemisia tabaci* Genn. (Vasudeva and Samraj, 1948; Cozosnek *et al.*, 1988; Chakraborty *et al.*, 2003).

Most of investigations carried on this disease are confined to symptomatology, transmission and physiological identification (Yassin and Nour, 1965). Generally virus infection is known to result in drastic histopathological changes. The effects of Tomato Leaf Curl Virus on the anatomic structure of plants have received less attention, than comparable effects on plants infected with leaf curl virus. Therefore, the aim of present investigation is to analyse the anatomical changes of tomato roots, stems and leaf affected by TLCV.

Materials and Method

In March 2006, tomato plants were raised under green house condition. For transmission of TLCV, the adult whiteflies (*Bemisia tabaci* Genn.) were collected from TLCV infected tomato plants. These whiteflies were then fed on infected tomato plants for 48 hrs in an insect proof cage to ensure the acquisition of virus. Later 20 whiteflies were removed and transferred to each healthy tomato plant kept in an insect proof cage in three replications. An equal number of plants were kept in green house as uninoculated controls.

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Leaves, stems and roots were sampled from infected and healthy plants and fixed in FAA after 24 hrs, washed in 70% alcohol. The plant material were dehydrated with ethyl alcohol butanol grades and embedded in paraffin wax. Sections were cut on rotary microtome at 10µm thickness. These sections were stained with safranin & fast green and mounted in DPX (Johansen, 1940). The stomatal size, frequency and stomatal index were measured with a precalibrated stage & ocular micrometer. The stomatal index (SI) was calculated (Salisbury, 1928).

$$SI = \frac{\text{No. of stomata per unit area}}{\text{No. of stomata per unit area} + \text{No. of epidermal cell}} \times 100$$

Results and Discussion

Symptoms- Plants of tomato infected with TLCV were severely stunted, leaf curling and shrinking of leaves. Leaves were often bent downwards or upwards and were stiff. Fig. A to G shows T.S. of leaves and stems of infected and healthy plants.

Anatomical observations in root- The cells of epiblema were smaller in size & possessed lignified walls on its inner surface. The cortical and endodermal cells of diseased plants were relatively small with thicker walls. The pith region had large cells in middle region with less developed conducting tissue, viz; phloem and xylem (Fig. A). Almost similar observations had also been made in tomato root infected with TMV. The epidermal cells were smaller in size and had large pith region (Dubey *et al.* 1982). The urdbean leaf crinkle virus caused reduction in cell size of phloem and xylem in urdbean. Epiblema, cortex endodermis, medullary rays and pith were found thickened (Sharma and Dubey, 1985).

Anatomical observations in stem- In diseased stem, the xylem vessels were fewer narrow and reduced in number. Phloem necrosis was observed in infected stem (Fig. B). The cortical parenchyma was wider and formation of mechanical and conducting tissues was reduced. The cell wall of the infected ground tissues was reduced in thickness. These results were found to be in conformity with the findings reported in groundnut infected with mosaic rosette virus (Singh, 1970). There was found to be marked reduction in the size of all the tissue of *Solanum khasianum* infected with green vein banding virus. Vascular bundles were reduced in sized and pith region greatly affected (Garg *et al.*, 1977). The cortical region was enlarged in tomato stem infected with TMV (Dubey and Bhradwaj, 1982).

Anatomical observations in leaves- Epidermal cells and the tissues of the midrib region especially on dorsal surface of infected leaf were degenerate (Table-1). The development of midrib was irregular. In vascular region the xylem parenchyma, xylem vessels and phloem were greatly reduced. Leaf tissue consisted in replacement of the spongy parenchyma by a palisade parenchyma (Fig. C). The palisade cells of diseased leaves were thinner (Table-2) & less densely packed with chloroplast. The significant changes were shown in spongy tissues. The average stomatal frequency and stomatal index were significantly lower in diseased leaves, although their size was increased (Table-3). The chloroplasts were few and showed irregular shape. The epidermal, palisade and spongy cells reduced in *Vicia faba* infected by tomato ring spot virus (TRSV) (Smith and Mc Whorter, 1957). The leaf tissues consisted in replacement of the spongy parenchyma by palisade parenchyma and cambial activity was observed in the main phloem parenchyma of *Athea rosea* infected with Begmovirus (Bigare *et al.* 2001). In vascular region the

phloem, xylem parenchyma and xylem vessels were greatly influenced in diseased tomato leaves with TMV (Dubey and Bhardwaj, 1982). The changes in colour as primary symptoms due to chlorosis seems to be due to the disintegration and disruption of chloroplasts in tobacco leaves infected by tobacco mosaic virus (Carrol and Kosuge, 1969).

Table 1: Thickness of epidermal cells of healthy and diseased leaves (mean of three replicate)

S.No.	Healthy		Diseased	
	Upper epidermal cell+ cuticle (μm)	Lower epidermal cell+ cuticle (μm)	Upper epidermal cell+ cuticle (μm)	Lower epidermal cell+ cuticle (μm)
1	51.25	43.10	38.35	35.10
2	50.50	41.32	36.20	32.26
3	50.10	40.25	35.66	31.25
4	49.41	40.20	35.43	30.45
5	49.25	40.10	34.50	30.40
6	46.50	39.80	34.12	30.10
C.D at 5%	4.35	7.28	2.51	4.8

Table 2: Thickness of palisade cells of healthy and diseased leaves (mean of three replicate)

S.No.	Thickness in μm	
	Healthy	Diseased
1	143.50	136.68
2	142.75	132.45
3	141.75	132.32
4	141.20	130.34
5	140.61	128.25
6	140.50	120.10
C.D at 5%	1.52	2.28

Table 2: Thickness of palisade cells of healthy and diseased leaves (mean of three replicate)

S.No.	Healthy Size of stomata (μm)				Diseased Size of stomata (μm)			
	SF	Length	Breath	SI	SF	Length	Breath	SI
1	617	17.95	9.18	26.07	483	23.63	14.45	31.4
2	589	17.85	9.03	25.74	461	21.93	12.75	30.92
3	578	17.34	7.65	25.54	440	21.42	12.11	29.86
4	557	16.83	7.65	25.52	428	21.42	11.47	28.86
5	515	15.30	7.65	25.15	373	20.90	10.83	27.93
6	419	15.00	7.65	24.94	367	20.40	10.20	27.84
7	431	14.00	7.30	24.28	317	20.20	9.60	21.53
C.D at 5%	42.37	1.38	0.92		30.18	2.56	1.84	

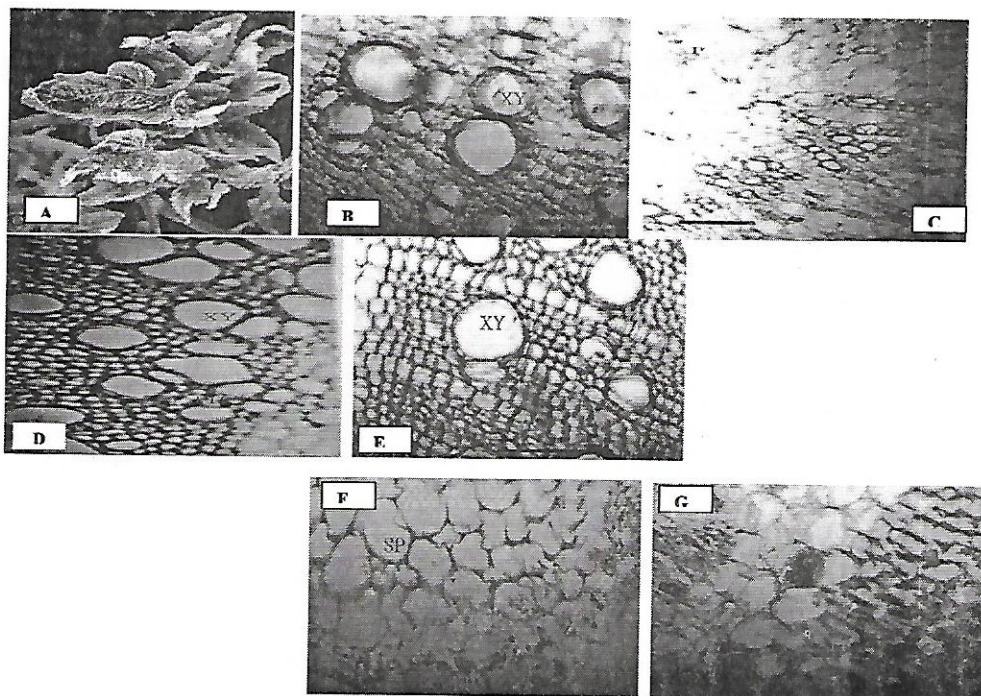


Fig. A : Tomato leaves infected with TLCV.

Fig. B : T.S. of healthy root.

Fig. C : T.S. of diseased root showing large pith region (P) & reduced xylem (XY), phloem (PH)
Bar : 130 μ m

Fig. D : T.S. of Healthy stem; Bar : 110 μ m

Fig. E : T.S. of diseased stem showing enlarge reduced xylem (XY), necrosis of phloem (PH).

Fig. F : T.S. of health leaf; showing spongy parenchyma (SP).

Fig. G : T.S. of diseased leaf showing degenerating epidermal cells (EP) & thin and less densely palisade parenchyma (PP).

Acknowledgement

The authors thank to the Head, Department of Botany, M.L.K.P.G. College, Balrampur (U.P.) for providing library and laboratory facilities. Thanks are also due to Dr. D. S. Shukla and Miss. Anjana Shukla for their help during experimentation.

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