GAU Res. J. 1980. 5 (2) : 20-24

Studies on Transmission of Different Types of Tobacco Leaf Curl Virus*

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ABSTRACT

The study of host-parasite and virus-sector relationship through transmission tests on different types of Tobacco leaf curl virus (TLCV) revealed that (i) the period for appearance of symptoms decreased when young tender leaves were inoculated with viruliferous white flies. (ii) The viruliferous white flies require a minimum period of 30 minutes or less, while the maximum being 8 hours for transmission of TLCV. (iii) The per cent infection increases with the increase in inoculation feeding period of the vector (*Bemisia tabaci*, Genn.)

(*Received on May 6, 1978*)

INTRODUCTION

Among the several hazardous viral diseases, leaf curl of tobacco (TLC) is the most destructive disease which causes quantitative as well as qualitative losses to the tobacco crop. This disease was first reported from Indonesia by Peters and Schwartz (1912) and from India by Pal and Tandon (1937).

TLC is caused by different strains of virus and the plant expresses varying symptoms according to the strain of virus involved (Pal and Tandon, 1937). The symptoms exhibited by these strains are also morphologically different. In nature, TLC is transmitted by a common vector white fly (*Bemisia tabaci*) (*B. gossypiperda*), Family-Aleyrodidae) as reported by Pruthi and Samuel, (1937, 1939). Hill (1968) successfully transmitted tobacco leaf curl disease to healthy test plant in the glass-house using *Bemisia tabaci* as vector. *Bemisia tabaci* is a common white-fly prevalent in Gujarat and hence, it is desirable to determine the role played by this insect in the development of different types of TLC in Gujarat. To determine the host-parasite and virus-vector relationship through transmission tests on different types of TLC, the present investigation was undertaken.

MATERIALS AND METHODS

Virginia gold, a highly susceptible, variety was grown in insect proof glass house and was used as host for

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maintaining the virus cultures of TLC, while *Nicotiana glauca* was used as host for multiplication of white flies. To maintain colonies of non-viruliferous white-flies, the first instar nymphs (crowler) were transferred to *N. glauca* seedlings grown in insect proof cages. For transmission test a microcage suggested by Capoor and Varma (1950) was used with slight modification. The microcage used for the study consisted of 5 cm long transparent polypropelene tube open at both ends with inner diameter of 2.2 cm. One of the ends of the tube was closed with a circular (3.0 cm diameter) corksheet, held in position with the tension of the galvanize wire spring. The other end of the tube was closed by tying a piece of black muslin cloth with a rubber band. An aspirator was used to release known numbers of white flies in to the microcage.

Plants affected with different types of TLC were selected on the basis of the symptoms described by Pal and Tandon (1937) and graded as type 'A', 'B', 'C', 'D' or 'X'. These strains were transferred individually from field to glass house on seedlings of virginia gold by the transmission technique. The inoculated plants showing typical symptoms were selected as the source of inoculum for further studies.

For transmission studies, non-viruliferous white flies were collected from the pure cultures with the help of an aspirator and were transferred to the microcage from the open end. This end was then closed with the black muslin cloth. The white flies were kept for two hours in the microcage for pre-acquisition fasting. The cage was then fixed on the lower side of the leaf to allow the white flies to feed for six hour (acquisition feeding). For inoculation feeding, these white flies were transferred to healthy plants grown in the glass house and were allowed to feed on the lower side of the leaf for 24 hours. The microcage was then removed and the white flies were killed. The tobacco plants thus inoculated were transferred on the bench in the insect free glass house till the symptoms developed.

RESULTS AND DISCUSSION

Experiment I:

To determine the incubation period of TLCV in relation to tenderness of the tobacco leaves, five viruliferous white flies per microcage were transferred to 70 days old tobacco test plants for inoculation feeding. Number of days required after the inoculation of TLCV for the first appearance of the symptoms were recorded on three different positions of the leaves viz. upper, middle and lower on the test plant. The results are summarised in Table 1.

The data (Table 1) reveal that the period for appearance of symptoms decreased as the tenderness of the leaves increased. The upper most leaves required minimum incubation period, while the lower ones required maximum period, the middle leaves being intermediate. This means that the white flies were actively feeding on the upper most leaves which were more tender and juicy than the middle and

TABLE 1

Number of days required as incubation period in relation to tenderness of the leaves.

Leaf Position	*Test plants*						*Transmission*		*Average Number of*
							Rate	*Per cent*	
	1[a]	2[a]	3[b]	4[b]	5[c]	6[c]			*Days*
Upper	19	28	19	28	29	19	6/6	100	23.7
Middle	*	*	24	*	39	44	3/6	50	35.7
Lower	54	*	35	*	*	51	3/6	50	46.7

*Symptoms did not appear

a Date of inoculation—2/10/75

b Date of inoculation— 4/10/75

c Date of inoculation—7/10/75

the lower leaves. There are reports that younger leaves are more susceptible than old ones. Bawden (1964) reported that french bean leaves from 10 days old plants gave more infections following inoculation with tobacco necrosis virus. The 13-14 days old plants gave none. Schein (1965) found that bean leaves were low in susceptibility to TMV at time of unfolding but susceptibility increased to a maximum when the leaves were about one-third fully expanded. Present study has shown that the infection is maximum in younger leaves and confirms the earlier work.

Experiment II:

To determine inoculation feeding period for transmission of TLCV to tobacco, five viruliferous white flies per microcage were transferred to 65 days old test plant and allowed for varying feeding period viz., 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours and 8 hours.

TABLE 2

Transmission of TLCV at varying inoculation feeding period on tobacco test plants.

Inoculation Feeding period	*Date of Inoculation*	*Test plants*					*Transmission*	
		1	2	3	4	5	*Rate*	*Per cent*
30 minutes	11/10/75	*	*	*	17	13	2/5	40
1 hour	11/10/75	*	*	35	12	*	2/5	40
2 hours	11/10/75	17	*	8	20	*	3/5	60
4 hours	12/10/75	18	9	16	*	21	4/5	80
6 hours	12/10/75	27	16	20	24	*	4/5	80
8 hours	12/10/75	13	27	16	11	16	5/5	100

*Symptoms did not appear.

It is clear from the data that minimum inoculation feeding period required by the white flies to transmit TLCV on test plants was 30 minutes, the minimum period tried in the experiment. The transmission rate and per cent infection of TLCV disease increased with increase in inoculation feeding period and cent per cent infection was obtained in 8 hours feeding period. The rate of transmission increased with the increase in inoculation feeding period. Similar trend has been observed by Cohen and Nitzany (1966) in case of tomato yellow leaf curl virus, Costa and Bennett (1950) for Euphorbia mosaic virus and Varma (1959) for tomato leaf curl virus.

Experiment III:

With a view to determine incubation period for different types of TLCV and their rate of transmission, ten viruliferous white flies per microcage for each different type of TLCV were transferred to tobacco test plants for inoculation feeding.

TABLE 3

Incubation period (in days) after inoculation feeding and rate of transmission for different types of TLCV.

Types of TLCV	*Test plants*					*Transmission*		*Average Number of Days*
	1	2	3	4	5	*Rate*	*Per cent*	
A	20	45	27	*	17	4/5	80	27.3
B	25	56	28	27	*	4/5	80	34.0
C	20	25	*	28	25	4/5	80	24.5
D	19	18	20	*	*	3/5	60	19.0
X	49	*	45	22	*	3/5	60	38.7

*Symptoms did not appear.

Data summarised in Table 3 indicate that the white flies carrying the specific type of TLCV, when fed on test plants, produced the symptoms of same type of TLCV but the incubation period varied from type to type. Types 'C' and 'D' required minimum incubation period. while types 'A'. 'B' and 'X' required the maximum. The results are in confirmity with those reported by Pruthi and Samuel (1939). However, the incubation period as reported by them was slightly higher for different types of TLCV. This might be due to the seasonal variations. They had conducted the experiment during cold weather, October 1936 to February 1937, while the present study was conducted during October-November 1975, when the weather was comparatively warm.

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