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Virus - Vector Relationships of Yellow Mosaic Virus and Whitefly *(Bemisia tabaci)* in Ridge Gourd

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Yellow mosaic virus disease of Ridge gourd caused by begomovirus is a major constraint in cucurbits cultivation. Ridge gourd yellow mosaic virus (YMV) was transmitted to healthy ridge gourd plants only by the whitefly, *Bemisia tabaci* and not through sap inoculation. Even a single viruliferous whitefly was able to cause 20.00 per cent infection but 100 per cent infection was obtained when 10 whiteflies were used per plant. YMV disease was successfully transmitted through *B. tabaci* whitefly which were given 12 h of Threshold Acquisition access period (AAP) and inoculation period. The percentage of transmission increased with increase in both acquiisition and inoculation feeding periods. Ten adult *B. tabaci* whiteflies were sufficient to cause 100 % transmission and the whitefly retained the infectivity up to be 6-9 days.

Keywords: YMV, ridge gourd, whitefly, virus-vector relationships.

Ridge gourd (*Luffa acutangula* L. Roxb) popularly known as Kalitori or angled gourd and belongs to *Cucurbitaceae* family. Ridge gourd originated in India and it is cultivated in the tropics for its tender edible fruits both on commercial scale and in kitchen gardens throughout India. It is a popular vegetable both as spring summer and rainy season crop (Yawalkar, 1985). A large number of diseases and pests affect cucurbits at different stages of growth in India. These results in losses through reduction in growth and yield and are responsible for distortion and mottling of fruit, making the product unmarketable. Among viral diseases, yellow mosaic disease has been considered as an important limiting factor in cucurbits productivity (Seshadri, 1996). Begomoviruses have been reported to be the cause of yellow mosaic disease. Begomoviruses transmitted by the whitefly, *Bemisia tabaci* are wide spread in tropical and subtropical regions of the world, where they cause numerous diseases in dicotyledonous plants including cassava, pulses, vegetables, tobacco and cotton (Muniyappa and Veeresh, 1984; Harrison, 1985; Brown and Bird, 1992). More than 50 geminiviruses have been reported to be transmitted by whiteflies (Bedford *et al*., 1994; Markham *et al*., 1994).

Characteristic symptoms of ridgegourd yellow mosaic disease include extensive chlorosis and mosaic mottling on newly emerged leaves, vein banding and severe mosaic mottling. The most characteristic symptoms of the disease on *Luffa cylindrica* are leaf curling, yellow spot on the newly emerged leaves, chlorosis and mosaic (Tiwari *et al*., 2012). Begomovirus disease symptoms such as yellow mosaic, leaf curling, puckering and vein bending were observed on bitter gourd, pointed gourd, sponge gourd and pumpkin (Tiwari *et al*., 2012). In India, cucurbits found to be infected by two important begomoviruses viz., Squash leaf curl

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china virus (SLCCNV) (Singh *et al*., 2008) and *Tomato leaf curl New Delhi virus (ToLCNDV)* on pumpkin (Phaneendra *et al*., 2012), on bottle gourd (Sorhab *et al*., 2010), on sponge gourd (Sohrab *et al*., 2003) and on bitter gourd (Tiwari *et al*., 2010).

The YMV infected ridge gourd samples were collected, DNA was isolated using CTAB method and subjected to PCR using specific primers. The virus was cloned and sequenced. The sequence analysis shown that YMV is strain of Tomato leaf curl New delhi virus (ToLCNDV).

The virus - vector relationship of YMV and *B. tabaci* on ridgegourd has not been studied in detail. This paper reports about the transmission characteristics of YMV by *B. tabaci* on ridgegourd.

MATERIALS AND METHODS

Ridge gourd plant samples with characteristic symptoms of yellow mosaic disease was collected from naturally infected plants in the fields during survey. The virus was maintained on ridge gourd plants in an insect proof cages by frequent transfers from diseased to healthy ridgegourd plants through whitefly, *Bemisia tabaci* Genn. Healthy colonies of *B. tabaci* were maintained on Cotton (*Gossypium hirsutum* cv. varalakshmi) in insect proof cages and used throughout transmission studies.

Minimum number of *B. tabaci* required for transmission

To determine the number of *B. tabaci* required for the successful transmission of YMV, non-viruliferous *B. tabaci* were given an AAP of 24 hr on YMV -infected plant separately. Viruliferous whiteflies were then transferred to 8-10 days-old young healthy ridge gourd seedlings at the rate of 1, 3, 5, 10, and 20 per seedling separately, and 10 plants were inoculated in each treatment. After an IAP of 24 hour, whiteflies were killed by spraying 0.03 per cent Imidachloprid. The plants were kept in an insect-proof glasshouse for symptom expression and per cent transmission was recorded.

Acquisition access period (AAP)

The effect of different AAP on the rate of transmission of YMV was tested by allowing *B. tabaci* to feed for 30 min, 1,6,12 and 24h on YMV infected plants separately. After the prescribed AAP, the whiteflies were transferred on to 8-10 days-old healthy ridge gourd seedlings at the rate of 10 whiteflies per plant. For each treatment 10 ridge gourd plants were inoculated. After 24 hr of IAP, insects were killed by spraying 0.03 per cent Imidachloprid. Plants were kept in the glasshouse for symptom development.

Inoculation access period (IAP)

To determine the influence of different IAP on transmission of YMV, *B. tabaci* were allowed for a 24 hr AAP on YMV-infected plants separately. Viruliferous whiteflies were then transferred to 8-10 days old ridge gourd seedlings for IAP of 30 min, 1,6,12 and 24 hr at the rate of 10 per seedling. Ten plants were inoculated for each treatment. Whiteflies were then killed by spraying 0.03 per cent Imidachloprid and plants were kept in an insect-proof glasshouse for symptom development.

Incubation period in vector

To estimate the incubation period of YMV, the whiteflies were given a minimum acquisition access period of 1 h on infected Ridge gourd plant. Groups of 10 whiteflies were released on healthy ridge gourd seedlings after 30min, 1, 6, 12 and 24 hr inoculation access periods separately. Inoculated plants were kept in an insect proof glasshouse for symptom production.

Persistence of virus in vector

To determine the persistence of YMV in adult *B. tabaci*, the whiteflies were allowed for 24 h AAP on YMV infected ridge gourd plant. Then single whitefly was released on ridge gourd seedlings. The whiteflies were serially transferred to the healthy ridge gourd seedlings 24 h intervals until the insects were alive in each case. After each IAP the plants were sprayed with 0.03 per cent Imidachloprid and kept in insect proof cages.

RESULTS AND DISCUSSION

Determination of number of whiteflies required for virus transmission

To ascertain the minimum number of *B. tabaci* required for efficient transmission, different groups of whiteflies (eg.1, 3,5,10 and 20) per plant were used for virus inoculation. Plants were enclosed on test plants with AAP and IAP of 24h each. Single adult whitefly could able to transmit the yellow mosaic virus with 20 per cent efficiency. The transmission efficiency increased to 40 and 80

per cent when three and five whiteflies were inoculated to healthy ridge gourd plants, respectively. Transmission efficiency was 100 per cent with ten or more whiteflies per plant (Table 1).

Single adult whitefly has been found capable of transmitting begomovirus, but with low transmission efficiency. Number of whitefly per test plant required to be vary with the nature of the plant species. For plants with fleshy tender tissues required minimum of 5 adult whitefly per plant to cause 100 per cent infection. For woody nature plant species, 5 or more whiteflies required to get 100% virus transmission. The differences could be due to the vector feeding preference or the rate of virus multiplication in inoculated plants. These results indicates that the number of insects and

Table 1. Determination of minimum number of viruliferous indigenous whiteflies, *B. tabaci* required for transmission of yellow mosaic virus

No. of viruliferous whiteflies used for transmission	No of plants infected out of 10 inoculated plants	Per Cent transmission	No of Days taken for Symptom development
1	2	20	10-30
3	4	40	8 -20
5	8	80	8-15
10	10	100	8-15
20	10	100	8-15

Acquisition access period (AAP) : 24 hrs
Inoculation access period (IAP) : 24 hrs

Table 2. Effect of different Acquisition access periods (AAP) on transmission of ridge gourd yellow mosaic virus disease through indigenous whitefly, *Bemisia tabaci*

Period of aquisition	No of plants infected out of 10 inoculated plants	Per Cent transmission	No of Days taken for Symptom development
30min	2	20	8-20
1 hour	3	30	8-15
6 hour	6	60	8-15
12 hour	10	100	8-15
24 hour	10	100	8-15

Average no. of viruliferous whiteflies used per plant: 10
Inoculation access period (IAP) : 24 hrs

Table 3. Effect of different Inoculation access periods (IAP) on transmission of ridge gourd yellow mosaic virus disease through indigenous whitefly, Bemisia *tabaci*

Period of Inoculation	No of plants infected out of 10 inoculated plants	Per Cent transmission	No of Days taken for Symptom development
30min	2	20	8-23
1 hour	4	40	8-20
6 hour	6	60	8-15
12 hour	10	100	8-15
24 hour	10	100	8-15

Average no. of viruliferous whiteflies used per plant: 10
Acquisition access period (AAP) : 24 hrs

the transmission efficiency are positively correlated. Jayashree *et al.,* (1999) and Muniyappa *et al* .(2003), reported that single whitefly was able to transmitt PYVMV with 30 per cent efficiency, which increased to 60 per cent when three whiteflies were used and 100 per cent transmission with five or more viruliferous whiteflies per test plant. A minimum of 15 viruliferous B. tabaci per plant were required to achieve 100 per cent infection of CYVMV (Mandal, 1989) and cotton leaf curl virus (CoLCV) in cotton (Nateshan *et al*., 1996).

Acquisition access period

Studies on determination of effect of different acquisition access periods revealed that a minimum AAP of 30 min was necessary for whiteflies to aquire the YMV, which resulted in 20 per cent transmission. An AAP of 1h and 6h resulted in 30 and 60 per cent transmission, respectively. An AAP of at least 12h and 24h was required for 100 per cent transmission. The number of days taken for symptoms expression varied from 7 to 23 days depending upon period of acquisition. Results revealed that the percentage of transmission increased with the increase in AAP (Table 2).

Similar results were found that, a minimum of 30 sec was required by *B. tabaci* to transmitt begomovirus on pumpkin, PYVMV (Capoor and Ahmad, 1975). A minimum AAP of 5 min (Jayashree *et al*., 1999) and 30 min (Muniyappa *et al*., 2003) was necessary for whiteflies to aquire PYVMV which resulted in 8.3 and 20.0 per cent transmission, respectively. An AAP of 6 hour or more resulted in 100 per cent (Jayashree *et al*., 1999; Muniyappa *et*

Table 4. Determination of incubation period of yellow mosaic virus in indigenous Whitefly, *Bemicia tabaci*

Incubation period	No of plants infected out of 10 inoculated plants	Per Cent transmission	No of Days taken for Symptom development
30min	1	10	8-20
1 hour	3	20	8-15
6 hour	5	60	8-15
12 hour	10	100	8-15
24 hour	10	100	8-15

Average no. of viruliferous whiteflies used per plant: 10
Acquisition access period (AAP) : 24 hrs
Inoculation access period (IAP) : 24 hrs

Table 5. Persistence of yellow mosaic virus in viruliferous indigenous *Bemisia tabaci* [1]

No. of whiteflies / seedlings	Plant number	Serial transfer in days									
		1	2	3	4	5	6	7	8	9	10
1	1	+	+	+	+	+	+	D			
	2	+	+	+	+	+	+	+	-	D	
	3	+	+	+	+	+	+	+	_	D	
	4	+	+	+	+	+	+	+	-	D	
	5	+	+	+	+	+	+	D			
5	1	+	+	+	+	+	+	+	-	D	
	2	+	+	+	+	+	+	+	+	-	D
	3	+	+	+	+	+	+	+	-	D	
	4	+	+	+	+	+	+	-	+	-	D
	5	+	+	+	+	+	+	+	-	-	D

Bemisia tabaci [1] were given 24 h acquisition and inoculation access period each,
+ = Positive transmission, - =Negative transmission, D=Death of whiteflies

al., 2003). With increase in AAP, the percentage of insect becoming viruliferous increased, as a result the percentage of disease transmission also increased. However. Sohrab *et al*. (2013) reported that a minimum AAP required to transmit the virus was 60 min for both Luffa: Del and Pum:Del isolates.

Inoculation access period

A group of 10 viruliferous adult whiteflies were allowed for inoculation of yellow mosaic virus. The inoculation period ranged from 30 min to 24h. Viruliferous whiteflies required a minimum IAP of 30 min to achieve 20 per cent transmission efficiency. An IAP of 1h and 6h resulted in an increased transmission efficiency of 40 and 60 per cent, respectively. An IAP of 12h or more resulted in 100 per cent transmission. The days taken for symptoms expression varied from 8 to 23 days when 10 viruliferous whiteflies per plant were used depending upon the IAP. The results also indicated that percentage transmission increased with the increase of IAP (Table 3). Similar virus transmission characters reported for begomoviruses like CoLCuV (Ripper and George, 1965), BYVMV (Varma, 1952), TYLCV (Cohen and Nitzany, 1966).

The hundred per cent transmission of yellow mosaic virus in was obtained with an optimum AAP, IAP and number of whiteflies in 12 h, 12 h and 10 whiteflies respectively (Table 1, 2, & 3).

Incubation period of yellow mosaic begomovirus in vector

The whiteflies were allowed for a minimum AAP of 24h on YMV infected plant to determine the incubation period. Groups of 10 viruliferous whiteflies were released on 10 healthy ridge gourd plants after 30min, 1h, 6h, 12h and 24h of incubation period. After each incubation period, whiteflies were given 20h IAP. The results revealed that a minimum of 30 min incubation period, which resulted in 10 per cent transmission. An incubation period of 12h and 24h resulted in 100 per cent transmission. The results also indicated that the transmission efficiency increased with increase in incubation period (Table 4). Incubation period of 6 h was sufficient for successful transmission of beet pseudo yellows by greenhouse whitefly. *T. vaporarium* (Duffus, 1965) and similar reports were also found by Muniyappa and Reddy (1976) with HYMV and ToLCV (Reddy and Yaraguntaiah, 1981) and a latent period of 19 h was observed for SLCV in *B. tabaci* (Cohen *et al*., 1983). Three h minimum incubation period was required to transmit CoLCuV (Ripper and George, 1965).

Persistence of yellow mosaic begomovirus in vector

Experiments were conducted in two sets with groups of five and ten viruliferous whiteflies. Groups of five viruliferous whiteflies were serially transmitted to healthy ridge gourd plants at 24h interval (Table 5). The whiteflies retained and transmitted YMV successfully to all ridge gourd test plants on the first day after virus acquisition. The transmission was sporadic, thereafter, for one week and YMV persisted in whitefly for at least 7 days, after which all of the whiteflies had died. A similar sporadic transmission pattern was obtained with groups of 10 whiteflies per plant.

The whiteflies retained and transmitted YMV successfully to all ridge gourd test plants on the first day after virus acquisition. The transmission was sporadic, thereafter, for one week and YMV persisted in whitefly for at least 6 days, after which all of the whiteflies had died. A similar sporadic transmission pattern was obtained with groups of 10 whiteflies per plant. These results are in agreement with the report of Babitha (1996) and Muniyappa *et al.*, (2003) who stated that the maximum retension period of PYVMV was eight days in indigenous whitefly using 10 and 15 viruliferous insects. Similar persistence period was found with ICMV (Mathew and Muniyappa, 1991) compared to ToLCV that was retained in *B. tabaci* throughout its life period (Reddy and Yaraguntaiah, 1981).

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REFERENCES

1. Yawalkar, K. S. Vegetable Crops of India. (3rd Edition).Agric. Horticultural Publishing House. Nagpur. 440010. 1985; pp. 166-170.
2. Seshadri, V. S., Cucurbits. In: Vegetable Crops

in India. Naya Prakash, Calcutta, 1996; 496pp.
3. Muniyappa, V and Veeresh, G. K., Plant virus diseases transmitted by whiteflies in Karnataka. *Indian Acad. Sci.* (*Anim. Sci.*), 1984; **93:** 397-406.
4. Harrison, B. D., Advances in geminivirus Research. *Annu. Rew. Phytopathol.* 1985; **23**: 55-82.
5. Brown, J. K. and Bird, J., Whitefly transmitted diseases in America and Caribbean Basin: past and present. *Pl. Dis.*, 1992; **76**: 220-225.
6. Bedford, I. D., Briddon, R. W., Jones, P., Alkaff, N. and Markham, P. G., Differentiation of three whitefly transmitted geminiviruses from the Republic of Yemen. *European J. Pl. Pathol.*, 1994; **100**: 243-257.
7. Markham, P. G., Bedford, I. D., Liu, S. and Pinner, M. S., The transmission of geminiviruses by *Bemisia tabaci. Pest. Sci.*, 1994; **42**: 123-128.
8. Tiwari, A. K., Snehi, S. K., Khan, M. S., Sharma, P. K., Raj, S. K. and Rao, G. P., Molecular detection and identification of Tomato leaf curl New Delhi virus associated with yellow mosaic and leaf curl disease of *Luffa cylindrica* in *India. Indian Phytopathol*., 2012; **65** (1): 80-84.
9. Singh, R., Raj, S. K and Prasad, V., Molecular characterizations of a strain of Squash leaf curl China Virus from North India. *J. Phytopathol*., 2008; **156**: 222–228.
10. Phaneendra, C., Rao, K. R., Jain, R. K. and Mandal, B., Tomato leaf curl New Delhi virus is Associated with Pumpkin Leaf Curl: A New Disease in Northern India. *Indian J. Virol.*, 2012; **23** (1):42–45
11. Sohrab, S. S., Mandal, B., Ali, A. and Varma, A., Chlorotic curly stunt: a severe begomovirus disease of bottle gourd in Northern India. *Indian J. Virol*., 2010; **21**: 56–63.
12. Sohrab, S. S., Mandal, B., Pant, R. P. and Varma, A., First report of association of *Tomato leaf curl New Delhi virus* with yellow mosaic disease of *Luffa cylindrica* in India. *Pl. Dis*., 2003; **87**: 1148.
13. Tiwari, A. K., Sharma, P. K., Khan, M. S., Snehi, S. K., Raj, S. K. and Rao, G. P., Molecular detection and identification of Tomato leaf curl New Delhi virus isolate causing yellow mosaic disease in Bitter gourd (*Momordica charantia*), a medicinally important plant in India. *Medicinal Plants*., 2010; **2**(2) :117-123.
14. Jayashree, K., Pun, K. B. and Doraiswamy, S., Virus-Vector relationships of yellow vein mosaic virus and whitefly (*Bemisia tabaci*) in pumpkin. *Indian Phytopathol*., 1999; **52**(1): 102-106.
15. Muniyappa, V., Maruthi, M. N., Babitha, C. R., Colvin, J., Briddon, R. W. and Rangaswamy, K. T., Characterisation of pumpkin yellow vein mosaic virus from India. *Ann. Appl. Biol.*, 2003; **142**: 323–331.
16. Nateshan, H. M., Muniyappa, V., Swanson, M. M. and Harrison, B. D., Host range, vector relations and serological relationships of cotton leaf curl virus from southern India. *Ann. Appl. Biol*., 1996; **128:** 233–244.
17. Capoor, S. P. and Ahmad, R. U., yellow vein mosaic disease of field pumpkin and its relationship with the vector, *Bemicia tabaci*. *Indian Phytopathol.*, 1975; **28**: 241-246.
18. Sohrab, S. S., Karim, S., Varma, A., Abuzenadah, A. M., Chaudhary, A. G., Damanhourimand, G. A. and Mandal, B., Characterization of Tomato Leaf Curl New Delhi Virus infecting cucurbits: Evidence for sap transmission in a host specific manner. *African J. Biotechnol.*, 2013; **12**(32) : 5000-5009.
19. Ripper, W. E. and Lioyod George, L., *Cotton pests of Sudan*. 1965; pp 90-106. Blackwell Publications, Oxford.
20. Cohen, S. and Nitzany, F. E., Transmission and host range of the tomato yellow leaf curl virus. *Phytopathol*, 1966; **56**: 1127-1131.
21. Duffus, J. E., Beet pseudo-yellow virus, transmitted by the greenhouse whitefly (*Trialeurodes vaporarum*). *Phyto pathol*., 1965; **55**: 479-480.
22. Muniyappa, V., Reddy, H. R. and Shivashankar, G., Yellow mosaic disease on horsegram. *Curr. Res*., 1976; **5**: 52-53.
23. Reddy, K. S. and Yaraguntaiah, R.C. Virus vector relationship in leaf curl disease of tomato. *Indian Phytopathol.*, 1981; **34**: 310-313.
24. Cohen, S., Duffus, J. E., Larsen, R. C., Lir, H. Y. and Flock, R. A., Purification, serology and virus vector relationship of squash leaf curl virus, a whitefly transmitted geminivirus. *Phytopathol*., 1983; **73**: 1669-1673.
25. Babitha, C. R., Transmission, host range and serodiagnosis of pumpkin yellow mosaic geminivirus. M.Sc (Agri.) Thesis, Univ. Agri. Sci., Bangalore, India, 1996; 76 pp.
26. Mathew, A. V. and Muniyappa, V., Transmission of Indian cassava mosaic virus by *Bemisia tabaci*. *Fitopatologia Brasileira*, 1991; **16**: 46-49.