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Bhavana S
M.Sc. Department of Plant Pathology, College of Agriculture, Shivamogga, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India

R Ganesha Naik
Department of Plant Pathology, College of Agriculture, Shivamogga, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India

BK Shivanna
Department of Plant Pathology, College of Agriculture, Shivamogga, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India

H Ravindra
Department of Agricultural Entomology, College of Agriculture, Shivamogga, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga

Corresponding Author:
Bhavana S
M.Sc. Department of Plant Pathology, College of Agriculture, Shivamogga, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India

Virus-vector relationship of ridge gourd yellow mosaic virus and whitefly *Bemisia tabaci*

Bhavana S, R Ganesha Naik, BK Shivanna and H Ravindra

Abstract
Ridge gourd yellow mosaic virus (RgYMV) is one of the virus affecting the ridge gourd (*Luffa acutangula*). It is transmitted by whitefly *Bemisia tabaci*. Minimum of ten viruliferous whiteflies were required to transmit the virus. However, hundred per cent transmission of RgYMV disease was observed when 10 whiteflies were released per plant. Twelve hours of acquisition access period (AAP) and inoculation access period (IAP) were required for the whiteflies to transmit the disease. The percentage of transmission increased with increase in both acquisition and inoculation period. A pre-acquisition starvation period of 2 h ensured hundred per cent transmission and the whitefly retained the infectivity up to 5-8 days.

Keywords: Ridge gourd yellow mosaic virus (RgYMV), *Bemisia tabaci*, AAP, IAP

Introduction
Ridge gourd (*Luffa acutangula* L.) is popularly known as ribbed gourd, angled gourd, Chinese okra, silky gourd and also kalitori. It is identified with its ridges and preferred as easily digestible vegetable. Since it is a vine crop, requires support for its growth and development. It is being cultivated in India, Egypt, China, Japan, South East Asia and parts of Africa (Nagarajaiah *et al*., 2015) [10]. India is considered to be the centre of origin of *Luffa* species, belongs to genus *Luffa* of cucurbitaceae family and has chromosome number of 2n=26. In India, it is cultivated on both commercial scale and in kitchen gardens during the spring-summer and rainy season (Karthik, 2017) [7]. Ridge gourd grown in an area of 24,800 hectare and has production of 3,16,925 MT (Anon, 2017) [1].
The crop is known to be affected by several plant diseases such as downy mildew (*Pseudoperonspora cubensis*), powderys mildew (*Sphaerotheca fulginea*), Pythium rot (*Pythium butleri*), collar rot (*Rhizoctonia solani*) (Gopalakrishnan, 2007) [4] and RgYMD (Patil *et al.*, 2017) [11] and thus limiting the crop yield. 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) [8 5]. Begomovirus disease symptoms such as yellow mosaic, leaf curling, puckering and vein bending, mottling, stunted growth of plant were observed on bitter gourd, pointed gourd, sponge gourd, squash, bottle gourd, gherkin and pumpkin (Tiwari *et al*., 2012; Rajeshwari *et al*., 2016) [21, 14].
In India, cucurbits found to be infected by two important begomoviruses *viz*., Squash leaf curl china virus (SLCCNV) (Singh *et al*., 2008) and Tomato leaf curl New Delhi virus (ToLCNDV) on pumpkin (Phaneendra *et al*., 2012) [12], on bottle gourd (Sorhab *et al*., 2010) [19], on sponge gourd (Sohrab *et al*., 2003) [18] and on bitter gourd (Tiwari *et al*., 2010) [20].
The virus - vector relationship of RgYMV and *B. tabaci* on ridge gourd was studied. This research article focus on the transmission characteristics of RgYMV (strain of ToLCNDV) by *B. tabaci* on ridge gourd.

Material and Methods
Ridge gourd plant samples with characteristic symptoms of yellow mosaic disease like yellowing, leaf curling, mosaic, brittling, mottling of leaves, crinkling, upward and downward curling of leaves, stunted growth was collected from naturally infected plants in the fields during survey. The virus was maintained on ridge gourd seedling (10-12 days) in an insect proof cage by frequent transfer from diseased to healthy ridge gourd plants through whiteflies, (*Bemisia tabaci*).

Healthy colonies of *B. tabaci* were maintained on Brinjal (*Saloanum* (*Saloanum meloginella*) (Butter and Rataul, 1997) in insect proof cages and used for transmission studies.

Minimum number of viruliferous whiteflies (B tabaci)

To determine the number of *B. tabaci* required for the successful transmission of RgYMV, non-viruliferous *B. tabaci* were given an Acquisition Access Period (AAP) of 24 h on yellow mosaic disease infected ridge gourd seedlings. Viruliferous whiteflies were then transferred to 8-10 days old young healthy ridge gourd seedlings at the rate of 1, 3, 5 and 10 (Hurakadli *et al.*, 2016) [6] per seedlings separately, and 10 seedlings were inoculated in every treatment. After an Inoculation Access Period (IAP) of 24 h, whiteflies were killed by spraying 0.03 per cent imidachloprid. The seedlings were kept in an insect-proof glasshouse for symptoms expression and per cent transmission was recorded. The seedlings were observed for expression of symptoms and per cent transmission was estimated by using the following formula (Rajanimala *et al.*, 2005) [15].

$$\text{Per cent transmission} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Acquistion access period (AAP)

The effect of different AAP on the rate of transmission of RgYMV was tested by allowing *B tabaci* to feed for 30 min, 1, 6, 12 and 24 h on yellow mosaic disease infected ridge gourd seedlings separately. Groups of 10 whiteflies were transferred onto 8 to 10 days-old healthy ridge gourd seedlings. For each treatment 10 ridge gourd plants were inoculated. After 24 h of Inoculation Access Period (IAP), whiteflies were killed by spraying 0.03 per cent imidachloprid. Seedlings were kept in the insect proof glasshouse for symptom expression.

Inoculation access period (IAP)

To determine the influence of different IAP on transmission of RgYMV, *B tabaci* were allowed for 24 h AAP on yellow mosaic disease infected ridge gourd plants separately. Group of 10 viruliferous whiteflies were then transferred to 8-10 days old ridge gourd seedlings for IAP of 30 min, 1, 6, 12 and 24 h. The ten seedlings were inoculated for each treatment. Whiteflies were then killed by spraying 0.03 per cent imidachloprid and seedlings were kept in an insect-proof glasshouse for symptom development.

Effect of pre-acquisition starvation period on the transmission of RgYMV

Effect of pre-acquisition starvation period on the transmission of RgYMV, whiteflies were allowed to starve for different period's *viz*., 30 min, 1 h, 2 h and 3 h. Then the whiteflies were given on AAP of 12 h and 24 h on yellow mosaic disease infected ridge gourd seedlings and released to one week old healthy seedlings at the rate of 10 viruliferous whiteflies. After 12 h and 24 h, the whiteflies were killed by spraying imidachloprid (0.03%). For each treatment, 10 seedlings were maintained. The plants were observed for the development of symptoms and per cent transmission was recorded.

Incubation period in vector

To estimate the incubation period of RgYMV, the whiteflies were given a minimum acquisition access period of 24 h on infected ridge gourd seedlings. Group of 10 whiteflies were released on healthy ridge gourd seedlings after 30 min, 1, 6, 12 and 24h incubation period separately. After each incubation period, whiteflies are given 24 h IAP. Inoculated plants were kept in an insect proof glasshouse for symptom expression.

Persistance of virus in vector

To determine the persistence of RgYMV in adult *B tabaci*, whiteflies were allowed for 24 h AAP on RgYMV infected ridge gourd seedlings. Then the group of one and five whiteflies in separate set of experiments was released on ridge gourd seedlings. The whiteflies were serially transferred to the healthy ridge gourd seedlings at 24h intervals until the whiteflies were alive in each case.

Results and Discussion

Determination of number of viruliferous whiteflies required for transmission of RgYMV

To know the minimum number of *B. tabaci* required for efficient transmission, varied number of whiteflies (1, 3, 5, and 10) per seedling was inoculated. Active transmission of 10 per cent was obtained when single whitefly was inoculated with 24 h AAP and IAP. The study revealed that the transmission efficiency increased from 20 to 70 per cent, respectively when three and five whiteflies were released into healthy ridge gourd seedlings. However, 100 per cent transmission efficiency was observed when ten whiteflies per seedling were released. This finding is in line with the (Hurakadli *et al.*, 2016) [6], where they reported 10 and 20 whiteflies were enough to cause 100 per cent transmission. On the other hand Rajinimala *et al.* (2005) [15] reported a minimum of 45 whiteflies were required for 100 per cent transmission.

Effect of Acquisition Access Period on transmission of RgYMV by B tabaci

A minimum AAP of 30 min was necessary for whiteflies to acquire the RgYMV, which resulted in 10 per cent transmission. An AAP of 1 h and 6 h resulted in 30 and 50 per cent transmission, respectively. An AAP of at least 12 h and 24 h was required for 100 per cent transmission. The number of days taken for symptoms expression varied from 8 to 10 days depending upon the period of acquisition. Results of this experiment revealed that the percentage of transmission increase with the increase in AAP. Similar results were obtained by Pramesh *et al.* (2013) [13] that the whiteflies required a minimum of 30 min AAP to transmit croton yellow vein mosaic virus.

Effect of Inoculation Access Period on transmission of RgYMV by B tabaci

Viruliferous whiteflies required a minimum IAP of 30 min to achieve 10 per cent transmission. An IAP of 1 h and 6 h resulted in an increased transmission efficiency of 40 and 70 per cent respectively. An IAP of at least 12 h or more resulted in 100 per cent transmission. The days taken for symptoms expression varied from 8 to 10 days when 10 viruliferous

whiteflies per seedling released depending upon the IAP. The results indicated that the percentage transmission increases with increase in IAP. Similarly, Patil *et al.* (2016) reported that minimum AAP and IAP for transmission of ridge gourd yellow mosaic virus by whitefly was 15 min.

Effect of pre-starvation period on RgYMV transmission

Generally pre-acquisition starvation period increased the transmission of RgYMV. A 2 h of pre-acquisition starvation period ensured 100 per cent transmission of RgYMV by the whiteflies. The pre-acquisition starvation period of 1h and 3h recorded a transmission of 60 and 80 per cent respectively. A minimum transmission of 20 per cent was obtained with 30 min pre-acquisition starvation period. Negative impact was observed when starvation period increased beyond 3 h. This finding is in concurrence with the reports of Rajanimala (2005) [15].

Determination of incubation period of RgYMV in indigenous whitefly, Bemisia tabaci

The whiteflies were allowed for a minimum AAP of 24h on RgYMV infected seedling to determine the incubation period. Group of 10 viruliferous whiteflies were released on 10 healthy ridge gourd seedling after 30 min, 1 h, 6 h, 12 h and 24 h of incubation periods. After each incubation period, whiteflies were given 24 h IAP. The results revealed that a minimum of 30 min incubation period resulted in 10 per cent transmission. An incubation period of 12 h and 24 h resulted in 100 per cent transmission. The results also indicated that the transmission efficiency increased with increase in incubation periods. Similar findings were observed by (Duffus, 1965) [3] that Incubation period of 6 h was sufficient for successful transmission of beet pseudo yellows by greenhouse whitefly (*T. vaporarium*) and Three h minimum incubation period was required to transmit CoLCuV (Ripper and George, 1965) [16].

Persistence of RgYMV in whitefly (Bemisia tabaci)

The experiments were conducted in two sets with groups of one and five viruliferous whiteflies. Group of one viruliferous whiteflies were serially transmitted to healthy ridge gourd seedling at 24h interval. The whiteflies retained and transmitted RgYMV (Begomovirus) successfully to all ridge gourd seedlings on the first day after virus acquisition. The transmission was sporadic thereafter, for one week and RgYMV persisted in whitefly for at least 5 days, after which all of the whiteflies have died. A similar sporadic transmission pattern was obtained with groups of five whiteflies per plant. These results are in agreement with the findings of Muniyappa *et al.* (2003) [9] who stated that the maximum retention period of PYVMV (Pumpkin yellow vein mosaic virus) was eight days in indigenous whitefly using 10 and 15 viruliferous whitefly. The similar results were obtained by Hurakadli *et al.* (2016) [6] with RgYMV *i.e.*, Maximum retention period was up to 6 to 9 days.

Table 1: Determination of number of viruliferous whiteflies (*B. tabaci*) required for transmission of RgYMV

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	1	10	10-25
3	2	20	8-20
5	7	70	8-15
10	10	100	8-10

AAP: 24h IAP: 24h Variety: Naga

Table 2: Effect of different Acquisition Access Period (AAP) on transmission of RgYMV through whitefly *Bemisia tabaci*

Period of acquisition	No. of plants infected out of 10 inoculated plants	Per cent transmission	No. of days taken for symptom development
30 min	1	10	8-20
1 hour	3	30	8-15
6 hour	5	50	8-15
12 hour	10	100	8-10
24 hour	10	100	8-10

Average number of viruliferous whiteflies per seedling: 10 IAP: 24h Variety: Naga

Table 3: Influence of pre-acquisition starvation period on transmission of RgYMV

SI. No	Pre-acquisition starvation period	Number of plants infected out of 10	Transmission
1	30min	2	20
2	1h	6	60
3	2h	10	100
4	3h	8	80

Average number of viruliferous whiteflies per plant: 10 IAP: 24h Variety: Naga

Table 4: Effect of Inoculation Access Period (IAP) on transmission of RgYMV through 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	1	10	10-23
1 hour	4	40	8-20
6 hour	7	70	8-15
12 hour	10	100	8-10
24 hour	10	100	8-10

Average number of viruliferous whiteflies per plant: 10 AAP: 24h Variety: Naga

Table 5: Determination of incubation period of RgYMV in indigenous whitefly, *Bemisia 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	10-20
1 hour	3	30	8-15
6 hour	5	60	8-15
12 hour	10	100	8-10
24 hour	10	100	8-10

Average number of viruliferous whiteflies per plant: 10 AAP: 24h IAP: 24h

Table 6: Persistence of Rg YMV in viruliferous indigenous whitefly (*Bemisia tabaci)*

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

Average number of viruliferous whiteflies per plant: 10 AAP: 24h Variety: Naga

Conclusion

The efficiency of RgYMV transmission by whiteflies in ridge gourd was determined by number of whiteflies inoculated per plant, acquisition and inoculation access periods and pre acquisition starvation periods. The rate of transmission was positively correlated with number of whiteflies, AAP, IAP and pre acquisition starvation period. The incubation period was inversely propotional to the number of whiteflies per plant, AAP, IAP and pre acquisition starvation period. Virus was transmitted by whiteflies in persistent manner with maximum retention period of 6 days.

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