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Molecular studies on transmission of mung bean yellow mosaic virus (MYMV) by *Bemisia tabaci* Genn. in Mungbean

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The whitefly *Bemisia tabaci* Genn. is an important pest worldwide because of its ability to cause damage by direct feeding and its role as a vector of plant viruses including geminiviruses. Yellow mosaic virus (MYV) is a serious disease of pulse crops including mungbean, blackgram, frenchbean, pigeonpea and soybean. Yellow mosaic diseases are one of the most important viral diseases in mungbean caused by mungbean yellow mosaic virus (MYMV) which lead to severe yield reduction and it necessitates development of MYMV resistant lines for improved crop yield. Basic studies were carried out to elucidate the characteristics of MYMV transmission by its vector, *B. tabaci*. Artificial transmission experiments with *B. tabaci* were conducted under greenhouse conditions using cylindrical nylon cages with wire mesh tops. After 24 h acquisition access period (ASP) on agroinfected mungbean plants, *B. tabaci* collected from these agroinfected mungbean plants were considered viruliferous and transferred to a separate cage with healthy mungbean plants as confirmed via agroinoculation. After 24 h inoculation access period (IAP), *B. tabaci* were removed and the plants were sprayed with an insecticide and kept for observations of symptom development for 10 to 25 days in insect cages. Studies concluded with mungbean accessions using ten whitefly adults with 24 h of ASP and IAP resulted in transmission of virus of 70.50, and percent in MYMVR 111 (*At* VA 221), MYMVR 29 (*At* VA 239) and MYMVR 29 (*At* VA 221) respectively. Ten viruliferous whitefly adults did not cause MYMV symptom in KMG 189 (*At* VA 221), ML818 (*At* VA 239) and MYMVR 57 (*At* VA 221). Twenty viruliferous whitefly adults were able to cause MYMV after 48 h ASP and 24 h IAP and resulted in the maximum transmission efficiency in MYMVR 55 (*At* VA 221) (85.00%) and MYMVR 55 (*At* VA 239) (83.50%). The virus was proven to be a persistent discrete fragment of 703 bp using the polymerase chain reaction method on viruliferous whitefly adults, while no bands were obtained from non-viruliferous *B. tabaci* adults reared on CO_2 brinjal host.

Key words: Mungbean yellow mosaic virus (MYMV), transmission, vector, *Bemisia tabaci*.

INTRODUCTION

The whitefly, *Bemisia tabaci* (Genn.) is one of the most economically important pests in many tropical and subtropical regions (Bock, 1982). This polyphagous pest can cause extensive damage in more than 500 species of agricultural and horticultural crops (Greathead, 1986) through its direct feeding, and its ability to directly transmit geminiviruses. Mungbean (*Vigna radiata* L.) is an important pulse crop in developing countries of Asia,

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Africa and Latin America where it is consumed as dry seeds or fresh green pods (Karuppanapandian et al., 2006). Mungbean serve as a vital source of vegetable protein (19 to 28%), mineral (0.18 to 0.21%) and vitamins. India is the leading mungbean producer, covering up to 55% of the total world acreage and 45% of total production (Rishi, 2009). Mungbean yellow mosaic virus belongs to family Geminiviridae (Fauquet et al., 2003). The family Geminiviridae is divided in to four genera, Mastrevirus, Curtovirus, Topocuvirus and Begomovirus (Ramos et al., 2008). Begomovirus is the largest genus of the family Geminiviridae (Dhakar et al., 2010) which is characterized by a pipartite genome or monopartite genomes that were transmitted in a circulative persistent manner by B. tabaci. Among biotic agents, plant viruses are responsible for a significant proportion of crop diseases (Prajapat et al., 2011). It causes serious economic losses in many major crops by reducing seed yield and quality (Kang et al., 2005). Yellow mosaic disease (YMD) is reported to be the most destructive viral disease, caused by yellow mosaic virus. Mungbean yellow mosaic virus causes severe yield reduction in all mungbean growing countries in Asia including India (Biswass et al., 2008). Among the various diseases, MYMV disease was given special attention because of its severity and ability to cause yield loss of up to 85% (AVRDC, 1998). Conventional methods are unsuccessful in developing MYMV resistant mungbean lines due to the lack of a reliable screening technique. Rogers et al., (1986) developed an innovative technique called “Agroinfection” which serve as an alternate route for viral infection of plants by using the Ti plasmid and was demonstrated in case of Tomato Golden Mosaic Virus. A new technique called agroinoculation was has been shown to be used successfully in screening. Agroinoculation was done using the viral constructs mobilized in *Agrobacterium tumefaciens* strains. This paper reports the molecular studies on transmission of Mungbean yellow mosaic virus (MYMV) by *B. tabaci* in mungbean and the development of a polymerase chain reaction-based technique to detect the virus from its insect vector.

MATERIALS AND METHODS

Mass culturing of *B. tabaci*

Field collected *B. tabaci* nymphs and adults were reared in insect cages containing thirty day old CO_2 brinjal plants to maintain a laboratory culture at the temperature 27 and relative humidity 70% for the studies three generation maintained and adult age two days after emergence and used for the experiments. *B. tabaci* needed for the insect transmission experiment were collected from the culture using an aspirator.

Acquisition access period of *B. tabaci* on MYMV agroinfected mungbean plants

Adult *B. tabaci* were collected from the laboratory culture with the help of an aspirator and transferred into a test tube which was then covered with muslin cloth. Adult whiteflies were starved for 2 h under cool conditions temperature at (24°C) at which time the mouth of the test tube was opened to allow the adults to transfer to MYMV agroinfected mungbean plants. Adult whiteflies 10 and 20 where then allowed to feed for an acquisition period of 24 and 48 h.

Agroinoculated mungbean plants

MYMV resistant agroinoculated mungbean plants were grown in pots and maintained in a greenhouse for future use in the transmission experiment. Need to describe conditions in the greenhouse (26°C and relative humidity 64.15%), media used to grow the plants, source of seed, watering regime, pot size. plant age at testing etc.

Insect (vector) transmission

The insect (vector) transmission protocol developed by Aidawati et al. (2002) was used. MYMV transmission experiments with *B. tabaci* were conducted using cylindrical nylon cages with mesh tops. Ten *B. tabaci* adults were introduced into the cage through a hole which was sealed afterwards. After 24 and 48 h acquisition access period, *B. tabaci* adults were removed from MYMV agroinfected mungbean plants and transferred to a separate cage containing healthy agro inoculated (resistant) mungbean plants. After 24 h inoculated access period *B. tabaci* adults were removed and the plants were sprayed with an insecticide (Dimethoate 30EC at 1 ml/L) and evaluated for MYMV symptom development 10-20 later. Five potted plants were used for each acquisition-access period and the percentage of virus infection was calculated from plants showing MYMV symptoms after 10-20 days. Resistance levels were assessed by visual scoring of symptoms under greenhouse conditions (26°C and relative humidity 64.15%), following the 1-9 grade scale for visual scoring of mungbean yellow mosaic virus diseases by Nene et al. (1981) (Table 1).

DNA extraction

Total nucleic acids were extracted from individual of viruliferous and non viruliferous whiteflies using CTAB (hexadecyl trimethyl ammonium bromide) method with necessary modifications. Quality and quantity of the isolated DNA was measured in Nanodrop® ND-1000 spectrophotometer (nanodrop technologies, USA) and 1.0% Agarose gel electrophoresis before being used as the template DNA for all polymerase chain reactions (PCR). The reagents were purchased from Bangalore Genei Ltd., Bangalore, India.

Detection of MYMV in *B. tabaci* by polymerase chain reaction

Adults of *B. tabaci* were collected after a 24 h acquisition access period. Sets of 10, 15, 20, 25 and 10 non-viruliferous *B. tabaci* were subjected to DNA extraction following the CTAB (hexadecyl trimethyl ammonium bromide) method of Goodwin et al. (1994), while DNA extraction from MYMV infected mungbean leaves was conducted using the method of Karuppanapandian et al. (2006). The method of Rojas et al. (1993) was used for amplification of viral DNA from *B. tabaci* extracts by polymerase chain reaction (PCR). The viruliferous nature of these insects were confirmed by polymerase chain reaction products amplified by viral coat protein gene specific primers. Individual insects of viruliferous *B. tabaci* in groups of 10, 15, 20, 25 and 10 non-viruliferous *B. tabaci*, MYMV infected mungbean leaf samples were taken for DNA extraction

Table 1. Grade scale for visual scoring MYMV diseases.

Grade	Description
1.	No visible symptom on leaves or very minute yellow specks on leaves
2.	Small yellow specks with restricted spread covering leaf area 0.1 to 5.0 %
3.	Yellow mottling of leaves covering leaf area 5.1 to 10%
4.	Yellow mottling of leaves covering leaf area 10.1 to 15%
5.	Yellow mottling and discoloration covering 15.1 to 30%
6.	Yellow mottling and discoloration covering 30.1 to 50%
7.	Pronounced yellow mottling and discoloration of leaves stunting of plants covering 50.1 to 75 %
8.	Severe yellow discoloration of leaves covering 75.1 to 90%
9.	Very severe yellow discoloration of leaves covering 90.1 to 100%

Table 2. Transmission efficiency of Mung bean yellow mosaic virus (MYMV) by *Bemisia tabaci* in Mungbean.

Name of the agroinoculated mungbean lines	Number of insects released per plant	Acquisition feeding period (Hours)	Inoculation period (Hours)	No. of days taken for symptom development	MYMV Infectivity (%)
KMG 189 (*At* VA 221)	10	24	24	-	No
ML818 (*At* VA 239)	10	24	24	-	No
ML 1108 (*At* VA 221)	10	24	24	-	No
MYMVR 29 (*At* VA 221)	10	24	24	16	65.00
MYMVR 29 (*At* VA 239)	10	24	24	16	69.00
MYMVR 57 (*At* VA 221)	10	24	24	-	No
MYMVR 95 (*At* VA 221)	10	24	24	-	No
MYMVR 95 (*At* VA 239)	10	24	24	-	No
MYMVR28 (*At* VA 221)	10	24	24	-	No
MYMVR28 (*At* VA 239)	10	24	24	-	No
MYMVR 111 (*At* VA 221)	10	24	24	15	70.50
SP 84 (VA221)	10	24	24	-	No
MYMVR 115 (*At* VA 221)	10	24	24	15	55.00
MYMVR 115 (*At* VA 239)	10	24	24	16	60.00
MYMVR 55 (*At* VA 221)	10	24	24	16	54.00
MYMVR 55 (*At* VA 239)	10	24	24	16	50.00
MYMVR80 (*At* VA 221)	10	24	24	-	No
MYMVR80 (*At* VA 239)	10	24	24	-	No
MYMVR 90 (*At* VA 239)	10	24	24	15	53.00
MYMVR90 (*At* VA 221)	10	24	24	-	No

NO - indicate that the plants did not show any visible symptom during observation period.

followed by PCR amplification along with DNA clone of *A. tumefaciens.* Amplified DNA fragments were electrophoresed in 1% agarose minigels in TBE buffer and detected with UV light after staining in ethidium bromide (Maniatis et al., 1982).

RESULTS

The results revealed that transmission experiment after a 24 h acquisition access and 24 h inoculation assess period ten *B. tabaci* viruliferous adults were able to cause transmission of virus up to 70.50, 69.00, 65.00, 60.00. 55.00, 54.00 and 53.00% in MYMVR 111 (*At* VA 221), MYMVR 29 (*At* VA 239), MYMVR 29 (*At* VA 221), MYMVR 115 (*At* VA 239), MYMVR 115 (*At* VA 221), MYMVR 55 (*At* VA 221) and MYMVR 90 (*At* VA 239), respectively (Table 2). Ten adults of viruliferous adults of *B. tabaci* did not cause MYMV symptom in KMG 189 (*At* VA 221), ML818 (*At* VA 239), MYMVR 57 (*At* VA 221), MYMVR 95 (*At* VA 221), MYMVR 95, MYMVR28 (*At* VA 221), MYMVR28 (*At* VA 239), MYMVR80 (*At* VA 221), MYMVR80 (*At* VA 239) and MYMVR90 (*At* VA 221). The control plants inoculated with non-viruliferous whiteflies did not show MYMV symptoms.

The results (Table 3) indicated that twenty viruliferous adults of *B. tabaci* did not cause YMV symptom after 48 h acquisition access and 24 inoculation assess period in KMG 189 (*At* VA 221), ML818 (*At* VA 239), ML 1108 (*At* VA 221), MYMVR 57 (*At* VA 221), MYMVR 95 (*At* VA 221), MYMVR 95 (*At* VA 239), MYMVR28 (*At* VA 221), MYMVR28 (*At* VA 239) SP 84 (VA221), MYMVR80 (*At* VA 221) and MYMVR80 (*At* VA 239). One entry namely MYMVR90 showed resistance against *At* VA 221 strain

Table 3. Transmission efficiency of Mung bean yellow mosaic virus (MYMV) by *B. tabaci* in Mungbean.

Name of the agroinoculated mungbean lines	Number of insects released per plant	Acquisition feeding period (Hours)	Inoculation period (Hours)	No. of days taken for symptom development	MYMV Infectivity (%)
KMG 189 (*At* VA 221)	20	48	24	-	No
ML818 (*At* VA 239)	20	48	24	-	No
ML 1108 (*At* VA 221)	20	48	24	-	No
MYMVR 29 (*At* VA 221)	20	48	24	15	75.00
MYMVR 29 (*At* VA 239)	20	48	24	16	70.00
MYMVR 57 (*At* VA 221)	20	48	24	-	No
MYMVR 95 (*At* VA 221)	20	48	24	-	No
MYMVR 95 (*At* VA 239)	20	48	24	-	No
MYMVR28 (*At* VA 221)	20	48	24	-	No
MYMVR28 (*At* VA 239)	20	48	24	-	No
MYMVR 111 (*At* VA 221)	20	48	24	16	80.50
SP 84 (VA221)	20	48	24	-	No
MYMVR 115 (*At* VA 221)	20	48	24	15	75.00
MYMVR 115 (*At* VA 239)	20	48	24	15	81.00
MYMVR 55 (*At* VA 221)	20	48	24	16	85.00
MYMVR 55 (*At* VA 239)	20	48	24	15	83.50
MYMVR80 (*At* VA 221)	20	48	24	-	No
MYMVR80 (*At* VA 239)	20	48	24	-	No
MYMVR 90 (*At* VA 239)	10	48	24	15	83.00
MYMVR90 (*At* VA 221)	20	48	24	-	No

NO - indicate that the plants did not show any visible symptom during observation period.

and it was found to be susceptible to *At* VA 239 strain. Twenty viruliferous adults of *B. tabaci* were able to cause MYMV after 48 h acquisition and 24 h inoculation assess period resulted the maximum transmission efficiency in MYMVR 55 (*At* VA 221) (85.00%), which was followed by MYMVR 55 (*At* VA 239) (83.50%), MYMVR 90 (*At* VA 239) (83.00%), MYMVR 29 (*At* VA 221) (75.00%) and MYMVR 29 (*At* VA 239) (70.00%). Twenty viruliferous adults of *B. tabaci* were did not cause MYMV symptom in KMG 189 (*At* VA 221), ML818 (*At* VA 239), MYMVR 57 (*At* VA 221), MYMVR 95 (*At* VA 221), MYMVR 95, MYMVR28 (*At* VA 221) and MYMVR28 (*At* VA 239), MYMVR80 (*At* VA 221), MYMVR80 (*At* VA 239) and MYMVR90 (*At* VA 221) after 48 h acquisition and 24 h inoculation assess period. Twenty whiteflies per plants were found to be effective for disease transmission. Typical symptoms appeared after a minimum incubation period of 24 h under green house condition. The control plants inoculated with non-viruliferous whiteflies did not show MYMV symptoms. The characteristics MYMV symptoms observed on naturally infected plants were appear in the form of small irregular yellow specs and spots along the veins, which enlarge until leaves were completely yellowed fewer flowers and pods that bear smaller, occasionally shriveled seeds in severe cases MYMV symptoms observed after 15 - 16 days of after virus inoculation.

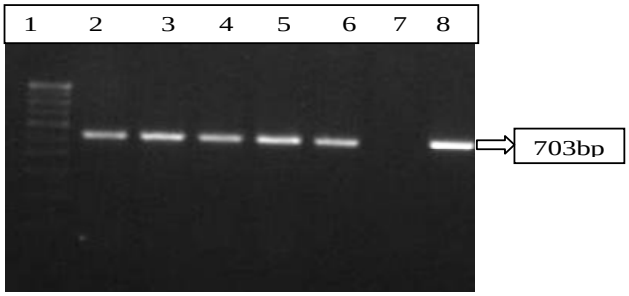

Figure 1. Polymerase chain reaction-amplified product of MYMV from viruliferous *B. tabaci*. Amplification of 703bp fragments with the primer pair coat protein forward and reverse primer (gene specific primers). A 15 µl aliquot of each polymerase chain reaction mixture was analyzed in a 1.0% agarose gel. Lane 1 - 100 bp ladder size marker. The samples are Lane 2) 10 individual of viruliferous *B. tabaci* with MYMV, Lane 3) 15 individual of viruliferous *B. tabaci* with MYMV, Lane 4) 20 individual of viruliferous *B. tabaci* with MYMV, Lane 5) 25 individual of viruliferous *B. tabaci* with MYMV, Lane 6) MYMV - infected mung bean, Lane 7) 10 individual of non viruliferous *B. tabaci*, Lane 8) DNA clone of *At* VA 239.

Detection of MYMV in *B. tabaci* by polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) amplified fragments of

the predicted size from the annealing positions of the coat protein (gene specific) primers were obtained from groups (10, 15, 20 and 25) of viruliferous *B. tabaci* and 10 non viruliferous *B. tabaci* (Figure 1). The virus was proven to be persistently discrete fragments of 703 bp were observed when polymerase chain reaction method was applied to detect the virus in viruliferous adults of *B. tabaci*, while no bands were obtained from non-viruliferous *B. tabaci* adults.

DISCUSSION

Geminiviruses are single-stranded DNA plant viruses with one or two circular genome components of 2.7 to 3.0 kb in size, encapsidated in twinned particles. They are transmitted by whiteflies. The whitefly species *B. tabaci* is the most efficient vector of members of the genus Begomovirus (1998; Van Regenmortel et al., 2000). Begomoviruses are currently emerging as a major threat in many tropical and subtropical regions in worldwide (Varma and Malathi, 2003). The probability of subsequent transmission of circulative viruses by insect vectors generally increases with increasing acquisition access period until all insects that are able to do so have acquired the virus (Swenson, 1967). Virus acquisition by insect vectors may depend on the virus titer in the infected plant, the ability of the insect to ingest the virus, and the passage of the virus through the midgut wall and subsequent survival in the insect vector. Transmission of MYMV was observed with ten adults of *B. tabaci* were able to cause transmission efficiency of virus up to 70.50, 69.00, 65.00, 60.00. 55.00, 54.00 and 53.00% in MYMVR 111 (*At* VA 221), MYMVR 29 (*At* VA 239), MYMVR 29 (*At* VA 221), MYMVR 115 (*At* VA 239), MYMVR 115 (*At* VA 221), MYMVR 55 (*At* VA 221) and MYMVR 90 (*At* VA 239), respectively (Table 2). The results reported by Aidawati et al. (2002) 100% tobacco leaf curl virus transmission efficiency occurred with a 24 h acquisition access period and inoculation access period. The results reported by Mehta et al. (1994) 24 h acquisition and inoculation access period was achieved maximum transmission efficiency with tomato yellow leaf curl virus.

After 48 h acquisition access period and 24 h inoculation access period resulted maximum transmission efficiency observed in MYMVR 55 (*At* VA 221), MYMVR 55 (*At* VA 239), MYMVR 90 (*At* VA 239), MYMVR 111 (*At* VA 221), MYMVR 29 (*At* VA 221) and ML 1108 (*At* VA 221) in 85.00, 83.50, 83.00, 81.00, 80.50, 75.00 and 70.00%, respectively (Table 3). Aidawati et al. (2002) reported that twenty *B. tabaci* adults cause 100% tobacco leaf curl virus transmission efficiency occurred with a 24 h acquisition access and inoculation assess period in tobacco. Transmission of begomoviruses from Indonesia by *B. tabaci* has been demonstrated earlier (Aidawati et al., 2002; Sudiono et al., 2001; Rusli et al., 1999).

The expected fragment of viral DNA 703 bp was amplified from a ten adult of viruliferous *B. tabaci* while no bands were obtained from non-viruliferous *B. tabaci* adults (Figure 1). Detection of MYMV from viruliferous *B. tabaci* using PCR technique showed that amount of viral DNA amplified in the polymerase chain reaction became shown higher as shown by the brightness of the DNA fragment in the gel electrophoresis. The results reported by Aidawati et al. (2002) tobacco leaf curl viral DNA fragments of 1.6 kb were observed when polymerase chain reaction method was applied to detect the virus in viruliferous nymphs and individual adults of *B. tabaci*, while no bands were obtained from non-viruliferous adults. The results were shown by Butter and Rataul (1977) with tomato leaf curl virus, as well as, Cohen and Nitzany (1966) and Mehta et al. (1994) with tomato yellow leaf curl virus. The virus persist inside the host genome the symptom development of 1-16 days after inoculation. The results reported by Cohen and Nitzany (1966) persistency of virus in the insect body varies, for example, 1-15 days for tomato yellow leaf curl virus, 8-55 days for tomato leaf curl virus (Butter and Rattaul, 1977).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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