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Host Range Studies for Yellow Mosaic Virus (YMV) Infecting Pulses

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ABSTRACT: *Pulses and grain legumes are major sources of dietary protein. These crops are subjected to yellow mosaic and golden mosaic diseases caused by white fly transmitted Gemini viruses (WTG's or begomovirus). Of these viruses, mungbean yellow mosaic India virus (MYMIV) is an important one. The virus reported from India is not mechanically transmitted but has been transmitted by the whitefly vector (Bemisia tabaci), not only to several species in the leguminosae but also to several weed species. Weeds may infect crops and those weeds may serve as a reservoir for crop-infecting geminiviruses .To investigate the potential begomoviral infection, infected weed samples were collected from the location of RARS ,Tirupathi, Andhra Pradesh. In the present report we identified a begomovirus infecting weeds like Sonchus arvensis, Acalifa indica, Acalifa celiyata, Hemidesmus indicus,, Rincozia minima, Carchorus olitorius, Crotton sparsiflorus, Tephrosia purpurea, Cida acuta, Aciranthes aspira, Carchorus trilocularis, Indigo species, Alysicarpus rugosus, Commilena benalensis, Boerhavia erecta, Cida rhombifolia, Rhincozia capiteta, Crossandra, Hyptis saveolensis, Euphorbia hirta , Abutilon indicum ,Phyllanthus niruri, Lantana camera, Acathosperum hispidum, Pedalium murex, Passiflora foetida, Vicia faba, Tridox procumbens, Vernonia cenraria, Digeria arvensis, Coccinia species, Euphorbia geniculata, Ageratum conyzoides, Duranta, Vigna trilobata, Andrographis echioids, Parthenium hysterophorus, Corchorus aestuans, Cleoma species, Trichodesma indicum, Ziziphus, Jatropha, calotrophis and Macroptilium.*

Keywords: *Host range, PCR, Weeds.*

INTRODUCTION

Pulses and grain legumes are major sources of dietary protein. These crops are subjected to yellow mosaic and golden mosaic diseases caused by whitefly transmitted geminiviruses (WTG's or begomovirus). There are two virus species causing YMD in grain legumes. They are the species Mungbean yellow mosaic virus (MYMIV) and Mungbean yellow mosaic India virus (MYMIV) and these viruses infects five major leguminous plants, such as blackgram, greengram, Frenchbean, pigeonpea and soybean causing an annual yield loss of about US $ 300 million (Varma *et al*., 1992).The virus reported from India is not mechanically transmitted but has been transmitted by the whitefly vector (*Bemisia tabaci*), not only to several species in the leguminosae (Nariani,1960) but also to *Brachiaria ramosa* (Gramineae) and *Cosmos bipinnatus*, *Eclipta alba* and *Xanthium strumarium* (Compositae) (Nene, 1973; Nene *et al*., 1971; Rathi and Nene, 1974) and several other leguminous weeds. The MYMV causes 85-100 per cent yield loss in the plants that are infected at the seedling stage (Nene, 1973). MYMV was first observed in Delhi in the late fifties (Nariani, 1960). Mungbean yellow mosaic virus is not seed borne this raises an important question regarding the survival of the viruses during the remaining nine months of non- cropping season and their subsequent transmission to the crops in the next cropping season. Gilbertson *et al*. (1991) performed dot-blot hybridisation analysis of a number of weeds in Dominican Republic that grew around bean fields and exhibited golden mosaic symptoms. General Geminivirus probes and probe specific to the Dominican Republic strain of bean golden mosaic virus (BGMV-DR) were used in the analysis. While a majority of the tested weeds harboured geminiviruses in general, only one weed *R. minima* may serve as a weed reservoir for BGMV-DR. On similar lines in order to find the perpetuation of MYMV, host range studies were carried out on weed species

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MATERIAL AND METHODS

The DNA was extracted from 45 weed species. The DNA was extracted by CTAB method (Murray and Thomson, 1980)). Infected plant material (0.5g) was ground in a pre-sterilized pestle and mortar with liquid nitrogen until a fine powder was obtained and transferred to sterile eppendorf tube. To this added 1ml of pre-heated (65°C) extraction buffer (100 mMTris (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% PVP, 0.1% Mercaptoethanal) and incubated for 1 hour in water bath at 65°C. Then tubes were centrifuged at 10,000 rpm for 10 min at room temperature and the supernatant was collected into eppendorf tubes. To this added equal volumes of chloroform and Isoamyl alcohol (24:1) and 1 µl RNase (100µg/µl) and incubated at room temperature for 10-20 min. Then centrifuged the tubes at 10,000 rpm for 10 min, separated the supernatant and added 0.1 volume of 3M sodium acetate (pH 4.8) and 0.6 volume of ice cold isopropanol then incubated at -20°C for overnight. After incubation, the tubes were taken out and centrifuged at 13,000 rpm for 20 min at 4°C. The supernatant was discarded and the pellet was washed with 70% alcohol and again centrifuged at 13,000 rpm at 4°C for 10 min, discarded the supernatant, air dried the pellets and dissolved in 100µl of sterile distilled water. The DNA samples were quantified by nano drop spectrophotometer and stored at -20°C for further use.

Polymerase Chain Reaction (PCR)

PCR was performed in 25µl reaction mixture using 1XPCR reaction buffer, 2.0Mm of $MgCl_2$, 0.2 mM of dNTPs, and 0.4µM of each primer(MYMIV-CP-500bp primer 5′ GGTCCCCTGATGTCCCTCGTG (forward) and 5′ ATGCGTTCTCAGTATGGTTCT (riverse)primer and MYMV MP-330bp primer 5′ TTGAATCTAATGTCTGTTGAGTG (forward) and 5′ AATTATGGGCAAATGCATGA(reverse), 1 U of DNA polymerase and 100ng of DNA template. The conditions for amplification of coat protein gene and movement protein gene are; 1 cycle of 95°C for 4min, 35 cycles of 94°C for 30s , 55°C for 1min ,72°C for 2min and 1 cycle of 72°C for 15min.

Analysis of PCR products by Agarose Gel Electrophoresis

Agarose gel electrophoresis of DNA was performed as described by Sambrook *et al*., (2001). The 1% agarose gel (W/V) was prepared by dissolving 0.5 g of agarose (Axygen, USA) in 50 ml of 1 X TBE buffer. The gel was allowed to cool for some time and then 2 µl of ethidium bromide (10 mg / ml) was added and poured into gel casting tray of mini horizontal electrophoresis unit (Hoefer, USA). The DNA samples were mixed with loading dye (Fermentas, USA) and the electrophoresis was carried in 1xTBE buffer at 50V (Labemate Power Pack 300, USA) till the dye front reached the lower part of the agarose gel. The migration pattern of the DNA fragments in the gel was recorded using gel documentation system (Alpha Innotech, USA) in an auto exposure mode.

RESULT AND DISCUSSION

Forty five weed species showing typical mosaic symptoms were collected from the fields of Regional Agricultural Research Station (RARS), Tirupati (Table.1). The DNA was isolated from 45 weeds and other crops showing yellow mosaic symptoms by CTAB method as described earlier (Fig. 1-7). The amount of DNA and purity of DNA (260/280 ratio) was measured in Nanodrop spectrophotometer. The concentration of DNA ranged from 240.44 ng/µl to 3991.8 ng/µl and 260/280 ratios ranged between 1.54 to 2.10. The extracted DNA gave good amplification of CP gene and MP gene in PCR when used at concentration of 100 ng/µl. The isolated DNA from 45 weed species is subjected to PCR amplification with MYMIV-CP-500 and MYMV-MP-330 primers and obtained 500bp and 330bp product respectively. About 45 weed species belonging to various families were tested by PCR using above two primers with negative control (blackgram healthy DNA) and positive control (YMV infected blackgram) and results were furnished in table 2.

The results show that MYMIV-CP-500 primer amplified 500bp product in 24 weed species. They are *Sonchus arvensis, Acalypha indica, Acalypha ciliata, Sida cardifolia, Croton sparsiflorus, Tephrosia purpurea, Sida acuta* (Fig. 8*), Indigo species, Alysicarpus rugosus, Commelina benghalensis, Sida rhombifolia* (Fig. 9), *Hyptis suaveolens, Euphorbia hirta, Boerhavia erecta, Lantana camara, Pedalium murex, Passiflora foetida, Vicia faba* (Fig 10), *Coccinia species, Euphorbia geniculata, Duranta erecta, Jatropha curcas, Calotrophis gigantia ,Cleome viscosa* (Fig. 11).

PCR amplification of 45 weed species with MYMV-MP-330 primer resulted amplification of 330bp product in 29 weeds. They are *Sonchus arvensis, Acalypha indica, Acalypha ciliata, Rhynchosia minima, Croton sparsiflorus, Tephrosia purpurea, Sida acuta* (Fig 12), *Indigo species, Alysicarpus rugosus, Commelina benghalensis, Boerhavia erecta, Sida rhombifolia* (Fig 13), *Hyptis suaveolens, Euphorbia hirta, Sida cardifolia,*

Lantana camera, Pedalium murex, Passiflora foetida, Vicia faba, (Fig 14), *Vernonia cinerea, Coccinia species, Euphorbia geniculata, Ageratum conyzoides, Duranta species, Andrographis echioids (*Fig 15), *Cleome viscosa, Corchorus aestuans, Jatropha curcus, Calotrophis gigantia* (Fig 16).

Rhynchosia minima, Vernonia cinerea, Ageratum conyzoides, Andrographis echioids, Corchorus aestuans are amplified with MYMV-MP-330 primer but not with the MYMIV-CP-500 primer. These results indicate that the above samples are infected only with MYMV but not MYMIV.

Some weed species like *Boerhavia erecta, Commelina benghalensis, Pedalium murex* and *Vicia faba* which do not show any field symptoms are tested by PCR with both primers and obtained positive results for presence of MYMIV and MYMV. These results show that MYMIV and MYMV can also survive as latent infection without showing symptoms. Symptomless infection caused by different viruses has been reported in many crops like mumgbean, blackgram, chillies (Biswas and Varma, 2001; Biswas *et al*., 2005; Biswas and Varma, 2000; Polston *et al*., 2006). The symptomless host serve as reservoir of acquisition and transmission of viruses to susceptible crop plants which in turn induce symptoms under suitable environmental conditions causing crop loss.

The notable contribution in the present study was identification of six new hosts for MYMIV and MYMV for the first time in the world. They are *Jatropha curcus, Calotrophis gigantia, Pedalium murex, Passiflora foetida, Hyptis suaveolens, and Euphorbia hirta* in Andhra Pradesh.

Aiyanathanan *et al*. (1998) transmitted the virus from *Acalypha indica* and *Croton sparciflorus* to mungbean. When transmission was done back to weeds only *Croton sparciflorus* picked up the infection, indicating that *Croton sparciflorus* act as reservoir of MYMV. But these results are variable with the report of Vanitharani *et al*. (1996) who performed nucleic acid hybridization with DNA-B probe of YMV with DNA of *Acalypha indica*, *Vernonia cinerea*, *Sida acuta*, *Croton sparciflorus* and found that no hybridization signal was observed indicating that they are not related. When same test was performed with ICMV-DNA-A probe, positive results were obtained in four weed species indicating that they are related to ICMV. Packialakshmi *et al.* (2010) reported association new begomovirus with *Vernonia cinerea* from Madurai and named it as *Vernonia yellow vein virus* (VeYVV) based on full length sequence homology. In the present study *V. cinerea* gave positive results with MYMV-MP-330 primer but not with MYMIV-CP-500 primer. Hence further studies on cloning and sequencing of full length genome is required to establish the identity of virus associated *V.cinerea* at Tirupati is same as reported at Madurai.

Association of MYMIV and MYMV was observed in the present study with *Sonchus arvensis* showing yellow vein symptom at Tirupati. Mubin *et al*. (2010) characterized components of a begomovirus disease complex associated with *Sonchus arvensis* that consists of a monopartite begomovirus along with multiple beta and alpha satellites in Pakistan. Prajapat *et al.* (2013) first time reported association of begomovirus with *Sonchus asper* and recorded 98% homology with CP gene of *Ageratum Yellow Vein Virus*-Gorakpur. There is need to obtain full length sequences and also look for satellites presence in *S. arvensis* reported in this study to establish the correct identity of begomovirus associated with this weed at Tirupati.

In the present study association of MYMIV and MYMV was observed with *Duranta erecta* showing leaf curl symptoms. Marwal *et al*. (2012) first time reported association of begomovirus infecting leaf curl disease with *Duranta erecta* in Rajasthan and named it as papaya leaf curl virus based on sequence homology of CP gene.

The DNA isolated from *Rhyncosia minima* showed amplification with the MYMV-MP-330 primer but not with MYMIV-CP-500 primer indicating that association of MYMV with *R. minima at* Tirupati. Jyothsna *et al.* (2011) identified and characterized a new begomovirus infecting leguminous weed *Rhynchosia minima* in India, for which the name Rhynchosia Yellow Mosaic India Virus is proposed based on full length sequence homology. In the present study amplification of *Ageratum conyzoids* DNA was obtained with MYMV- MP-330 primer but not with MYMIV-CP-500 primer. Xiong *et al.* (2007) identified distinct begomovirus species associated with *Ageratum conyzoids* in China and named it as Ageratum yellow vein China virus (AYVCNV). Association of MYMIV with *Ageratum conyzoides* was also reported in India based on RCA amplification and digestion with restriction enzymes (Naimuddin *et al*., 2014).

The objective of this study was to identify the weed host of YMV infecting pulses in Andhra Pradesh. Because weeds are reservoirs of begomoviruses that infect crop plants and act as ''melting pots'' that yield new viruses/virus strains by recombination and component exchange due to their frequently harbouring multiple viruses.

Table 1
Name of the weed samples collected for detection of YMV, their families and symptoms

S.No	*Name of weed*	*Family*	*Symptoms*
1	*Acalypha ciliata*	Euphorbiaceae	YM
2	*Acalypha indica*	Euphorbiaceae	YM
3	*Acanthosperum hispidum*	Asteraceae	YV
4	*Achyranthes aspera*	Amaranthaceae	YV
5	*Ageratum conyzoides*	Asteraceae	CY
6	*Alysicarpus rugosus*	Fabaceae	YM
7	*Andrographis echioids*	Acanthaceae	YV
8	*Boerhavia erecta*	Nyctaginaceae	NS
9	*Calotrophis gigantia*	Apocynaceae	YS
10	*Cleome viscosa*	Capparidaceae	NS
11	*Croton sparsiflorus*	Euphorbiaceae	CY
12	*Crossandra*	Acanthaceae	ULC
13	*Coccinia species*	Cucurbitaceae	YV
14	*Commelina benghalensis*	Commelinaceae	NS
15	*Corchorus aestuans*	Tiliaceae	YGV
16	*Corchorus olitorius*	Tiliaceae	YV
17	*Corchorus trilocularis*	Tiliaceae	YV
18	*Digera arvensis*	Amaranthaceae	CY
19	*Duranta erecta*	Verbanaceae	SG,ALC,CL
20	*Euphorbia hirta*	Euphorbiaceae	MY
21	*Euphorbia geniculata*	Euphorbiaceae	CY
22	*Hemidesmus indicus*	Apocynaceae	DYS
23	*Hyptis suaveolens*	Lamiaceae	YV,CY
24	*Indigo species*	Fabaceae	YGP
25	*Jatropha curcas*	Euphorbiaceae	MY, LC
26	*Lantana camara*	Verbanaceae	YV
27	*Macroptilium*	Fabaceae	MY
28	*Passiflora foetida*	Passifloraceae	YV
29	*Pedalium murex*	Pedaliaceae	NS
30	*Phyllanthus niruri*	Euphorbiaceae	CY
31	*Parthenium hysterophorus*	Astaraceae	YV
32	*Rhynchosia capitata*	Fabaceae	YGP
33	*Rhynchosia minima*	Fabaceae	YGP
34	*Sonchus arvensis*	Asteraceae	YV.
35	*Sida acuta*	Malvaceae	YV, CY
36	*Sida cardifolia*	Malvaceae	CY,ULC
37	*Sida rhombifolia*	Malvaceae	YV
38	*Tabernaemontana divaricata*	Apocynaceae	LC
39	*Tephrosia purpurea*	Fabaceae	YV
40	*Tridax procumbens*	Malvaceae	CY
41	*Trichodesma indicum*	Boraginaceae	YV
42	*Vicia faba*	Fabaceae	NS
43	*Vernonia cinerea*	Asteraceae	SG, ULC, YV
44	*Vigna trilobata*	Fabaceae	MY
45	*Ziziphus sps*	Rhamnaceae	CY

YM- Yellow Mosaic, YV- Yellow Vein , CY- Complete Yellowing, ULC- Up ward leaf curling, YGV- yellowing with green veins, SG- stunted growth, ALC- apical leaf curling, LC- leaf curling, MY- mild yellowing, DYS- dot like yellow spots, YGP- yellow and green patches, NS- no symptoms

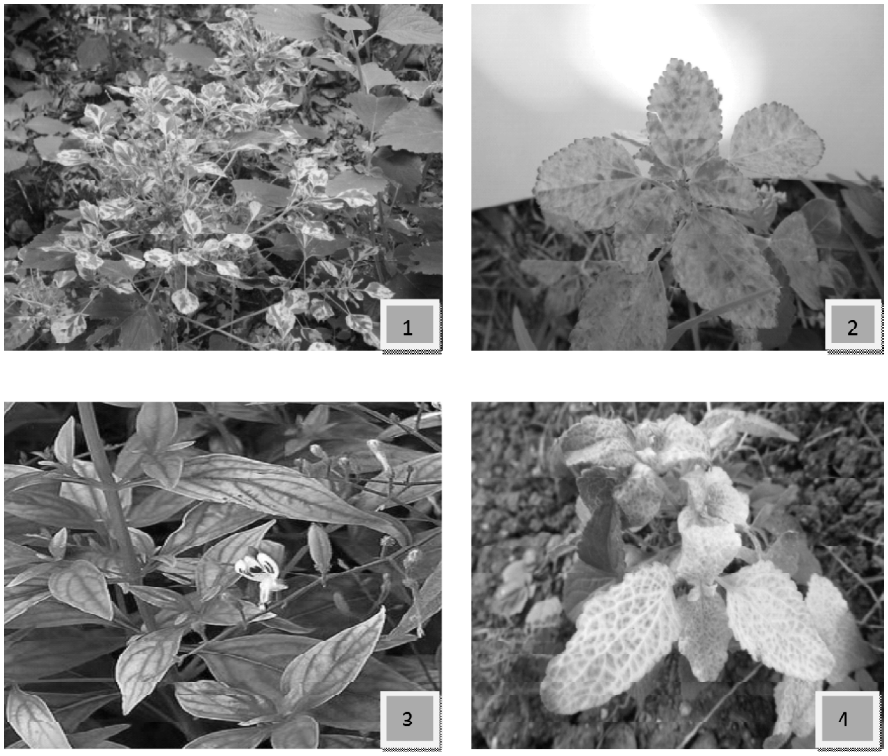

Figure 1: Weeds showing typical yellow mosaic symptoms

1. Acalypha indica, 2. Acalypha ciliata,
3. Andrographis echinoids, 4. Ageratum conyzoides.

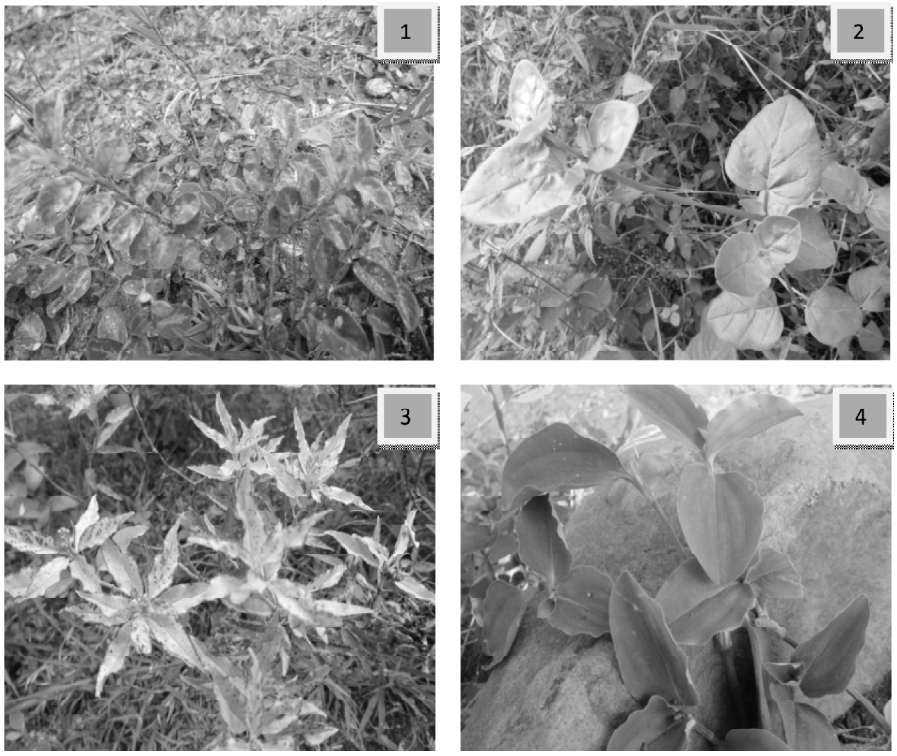

Figure 2: Weeds showing typical yellow mosaic symptoms
(1) Alysicarpus rugosus, (2) Boerhavia erecta
(3) Crotton sparsiflorus, (4) Commelina benghalensis.

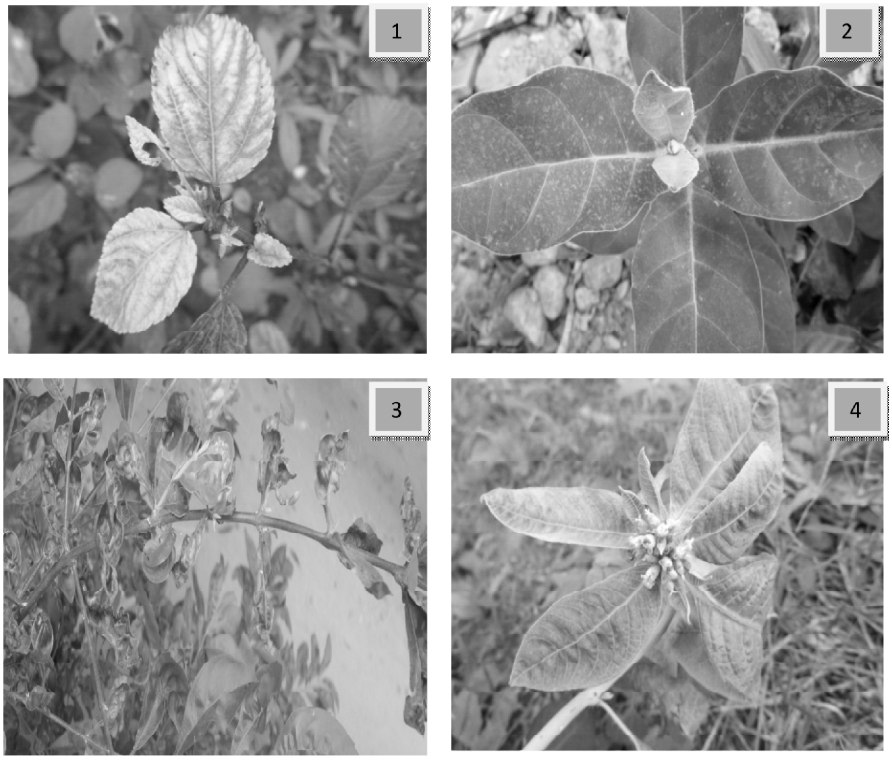

Figure 3: Weeds showing typical yellow mosaic symptoms

(1) Corchorus aestuans (2) Calotrophis gigantia
(3) Duranta erecta (4) Euphorbia geniculata

Figure 4 : Weeds showing typical yellow mosaic symptoms

(1) Euphorbia hirta, (2) Hyptis suaveolens,
(3) Indigo species, (4) Jatropha curcas

Figure 5 : Weeds showing typical yellow mosaic symptoms
(1) Lantana camera. (2) Pedalium murex,
(3) Rhincozia minima, (4) Passiflora foetida

Figure 6 : Weeds showing typical yellow mosaic symptoms

(1) Sida acuta, (2) Sida cardifolia,
(3) Sida rhombifolia, (4) Sonchus arvensis.

Figure 7 : Weeds showing typical yellow mosaic symptoms

(1) Vernonia cinerea, (2) Cleome viscose, (3) Vicia faba, (4) Tephrosia purpurea, (5) Coccinia spp

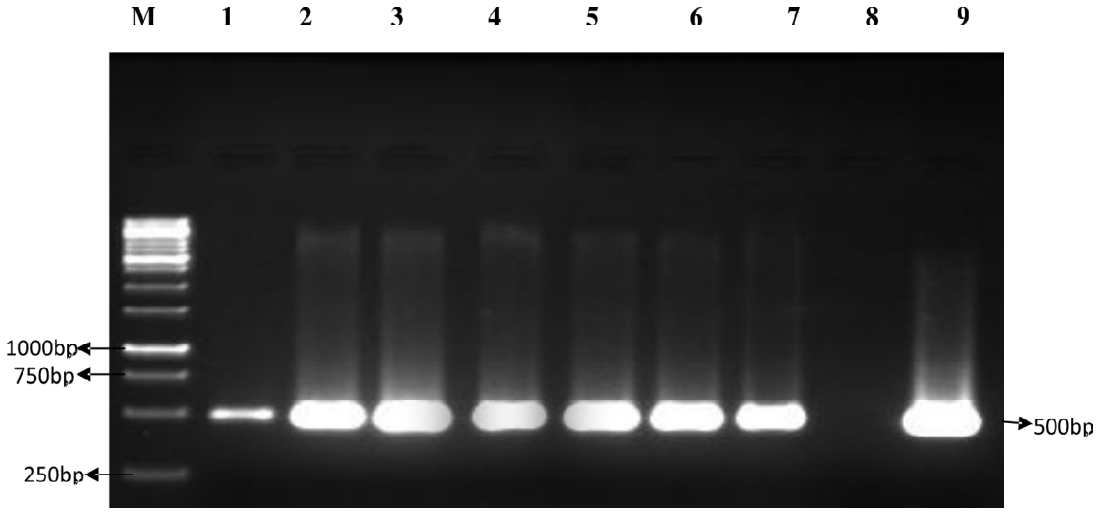

Figure 8: Detection of MYMIV-DNA-A in weeds by PCR with MYMIV-CP-500 primer.
Lanes: M.1Kb DNA ladder, 1.*Sonchus arvensis*, 2.*Acalypha indica*, 3.*Acalypha ciliata*, 4.*Sida cardifolia*, 5.*Crotton sparsiflorus*, 6.*Tephrosia purpurea*, 7*Sida acuta* 8.Negative control, 9.Positive control.

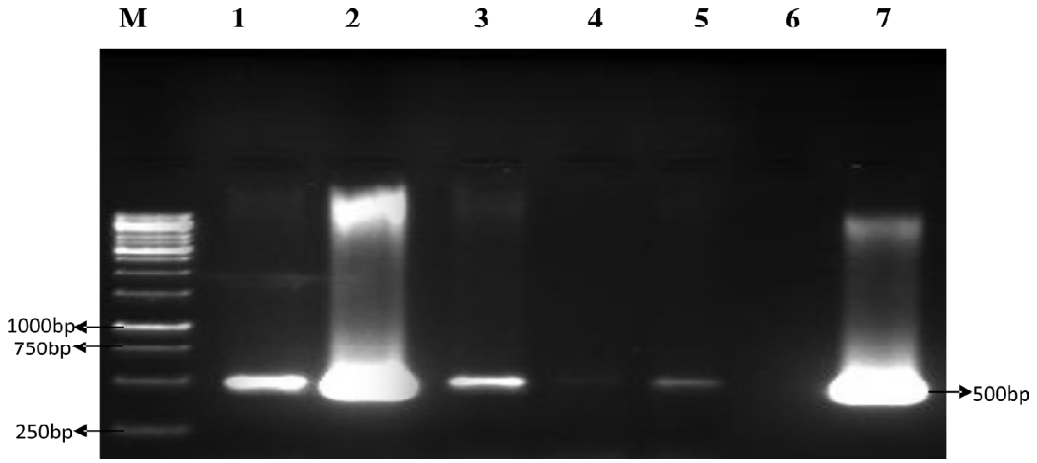

Figure 9 : Detection of MYMIV-DNA-A in weeds by PCR with MYMIV-CP-500 primer.
Lanes: M.1Kb DNA Ladder 1.*Alysicarpus rugosus*, 2.*Indigo species*, 3.*Commelina benghalensis*, 4.*Corchorus aestans*(no result here), *5.Sida rhombifolia* 6.Negative control, 7.Positive control,

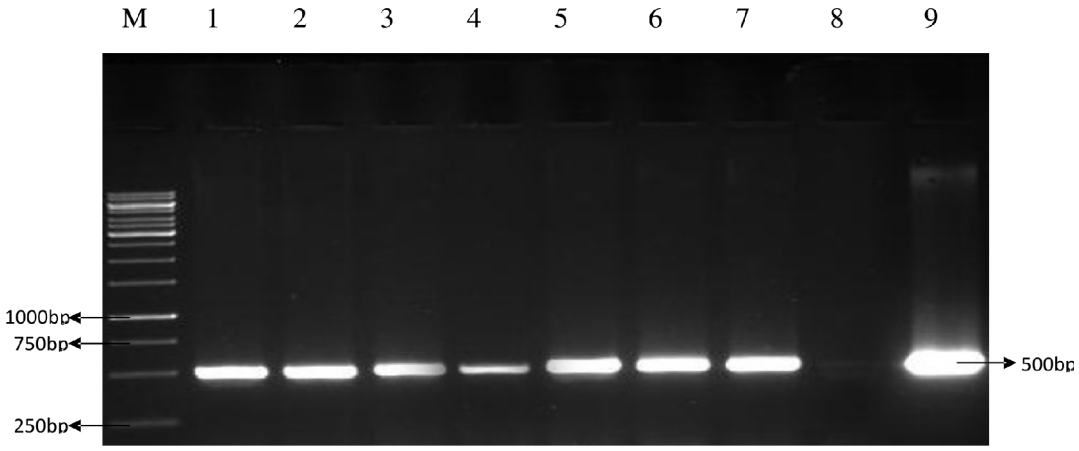

Figure 10: Detection of MYMIV-DNA-A in weeds by PCR with MYMIV-CP-500 primer.
Lanes: M. 1 Kb DNA Ladder, 1. *Euphorbia hirta*, 2.*Hyptis suaveolens*, 3.*Boerhavia erecta*, 4.*Lantana camera*, 5.*Pedalium murex*, *6.Passiflora foetida*, 7.*Vicia faba*, 8.Healthy plant, 9.Positive control.

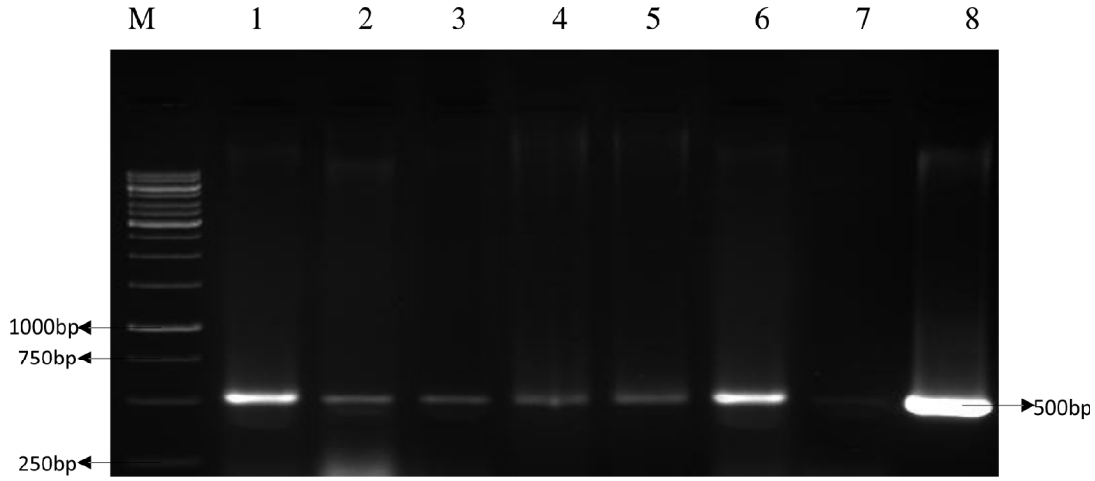

Figure 11: Detection of MYMIV-DNA-A in weeds by PCR with MYMIV-CP-500 primer.
Lanes: M.1Kb DNA Ladder, 1.*Coccinia species*, 2.*Euphorbia geniculata*, 3.*Duranta erecta*, 4.*Jatropha curcas*, 5.*Calotrophis gigantia*, 6.*Cleome - viscosa*, 7.Healthy DNA, 8.Positive control.

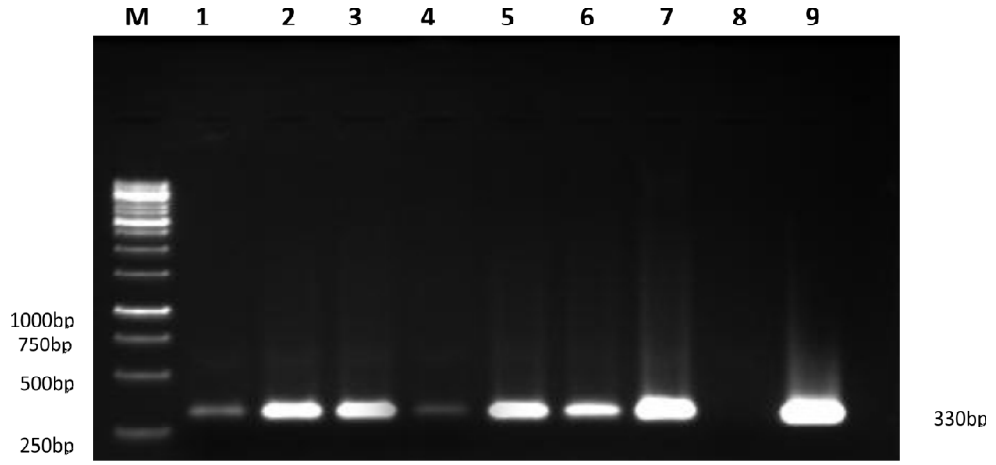

Figure 12: Detection of MYMV-DNA-B in weeds by PCR analysis with MYMV- MP-330bp primer
Lanes: M. 1Kb DNA ladder 1.*Sonchus arvensis*, 2.*Acalypha indica*, 3.*Acalypha ciliate*, 4.*Rhincozia minima*, 5.*Crotton sparsiflorus*, 6.*Tephrosia purpurea*, 7.*Sida acuta*. 8.Negative control, 9. Positive control,

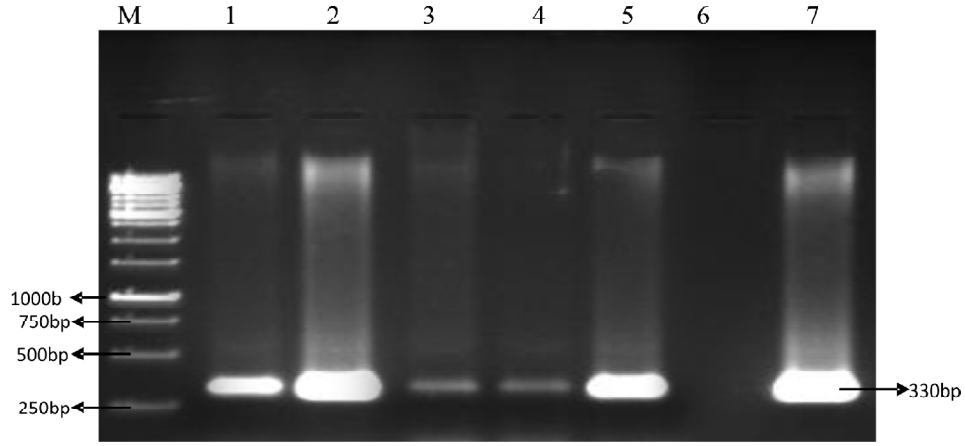

Figure 13: Detection of MYMV-DNA-B in weeds by PCR analysis with MYMV- MP-330bp primer
Laness: 1. *Indigo species*, 2. *Alysicarpus rugosus*, 3.*Commelina benghalensis*, 4. *Boerhavia erecta*, 5. *Sida rhombifolia*, 6.Negative control and 7.Positive control.

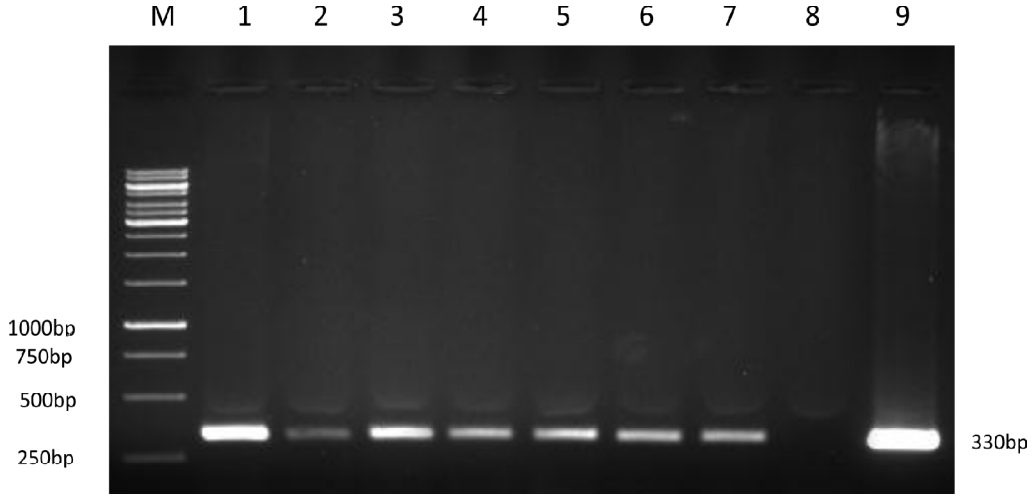

Figure 14 : Detection of MYMV-DNA-B in weeds by PCR analysis with MYMV- MP-330bp primer
Lanes: M.1Kb DNA Ladder, 1. *Hyptis suaveolens*, 2.*Euphorbia hirta*, 3.*Sida cardifolia*, 4.*Lantana camera*, 5.*Pedalium murex*, 6.*Passiflora foetida*, 7. *Vicia faba*,8.Negative control, 9. Positive control.

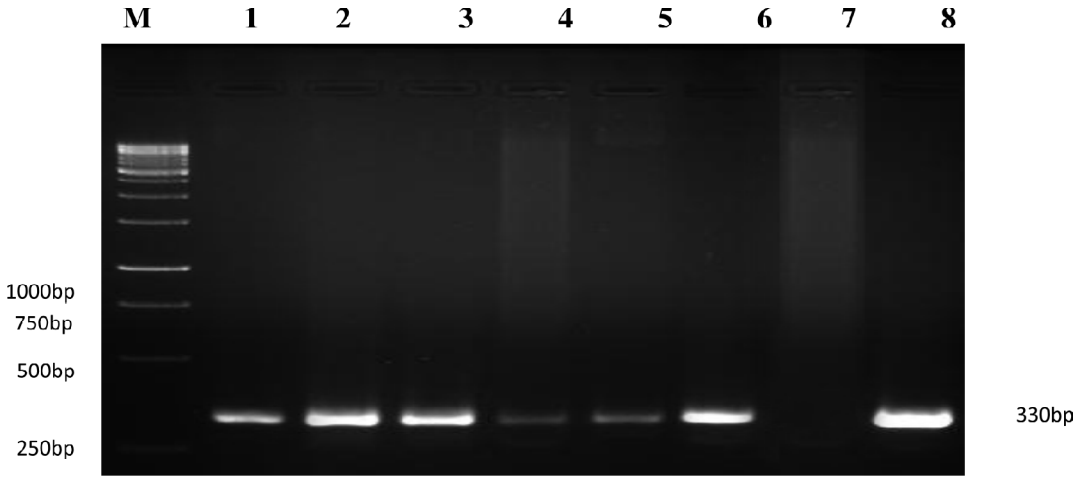

Figure 15: Detection of MYMV-DNA-B in weeds by PCR analysis with MYMV- MP-330bp primer.
Lanes : M. 1 Kb DNA Ladder, 1.*Vernonia cinerea*, 2.*Coccinia species*, 3.*Euphorbia geniculata*, 4. *Ageratum conyzoides*, 5.*Duranta erecta*, 6.*Andrographis echioids*, 7. Negative control, 8. Positive control.

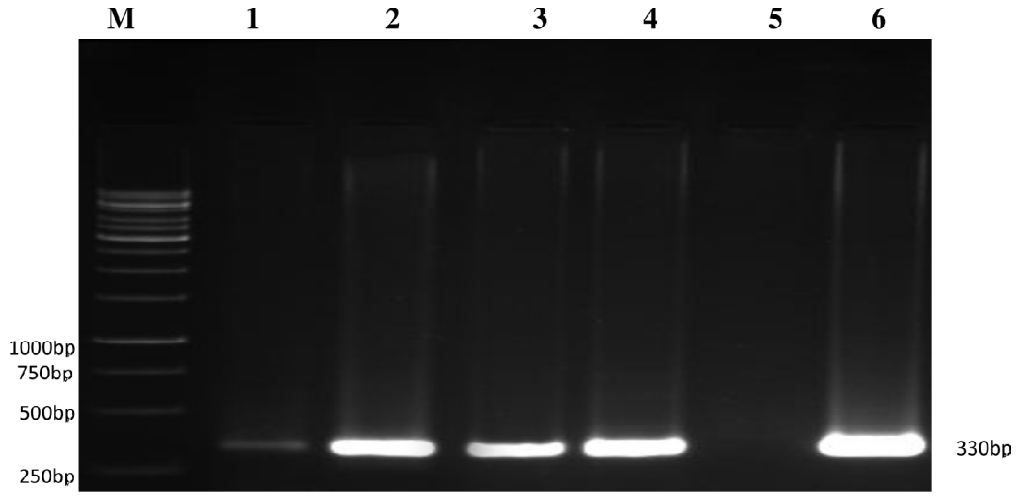

Figure 16: Detection of MYMV-DNA-B in weeds by PCR analysis with MYMV- MP-330bp primer
Lanes: M. 1Kb DNA Ladder 1.*Cleome viscosa*, 2. *Corchorus aestuan* 3. *Jatropha curcas*, 4.*Calotrophis gigantia*, 5.Negetive control, 6. Positive control,

Nevertheless, weeds have in the past been neglected in the study of diversity of plant viruses. Weeds acting as reservoirs can play an important part in the emergence of plant viral epidemics affecting crops. Weeds may serve as reservoirs of viruses during the non-cropping season. The possible recombination between weed and crop-infecting begomoviruses and their associated satellite DNA molecules may result in new viruses with wider host adaptations (Mubin *et al*., 2010; Xiong *et al.*, 2007; Packialakshmi *et al*., 2010). In this study we have identified several weeds as host for MYMIV and MYMV using PCR amplification of partial CP and MP genes, but there is need to clone full length sequences of both the components of MYMIV and MYMV and also look for association of alpha and betasatellites with these weeds to assign exact identity. Transmission studies from weeds to pulses and vice versa is also required to get information on exact role of them in spreading disease under field conditions.

FUTURE LINE OF WORK

1. Complete characterization of begomovirus and satellites associated with weeds and its role in spreading disease in the field to be studied.

REFERENCES

Aiyanathan, K. E. A., Chandrasekaran, A. (1998), Studies on weed hosts of mungbean yellow mosaic virus. *Indian Journal of Virology*. 14: 125-126.

Table 2
Detection of MYMIV and MYMV in weeds by PCR with primers YMV- CP 500 and MP-330 primers

S.No	Sample	Primers	
		MYMIV-DNA-A	MYMV-DNA-B
1	*Acalypha celiyata*	+	+
2	*Acalypha indica*	+	+
3	*Acanthosperum hispidum*	-	-
4	*Achyranthes aspera*	-	-
5	*Ageratum conyzoides*	-	+
6	*Alysicarpus rugosus*	+	+
7	*Andrographis echioids*	-	+
8	*Boerhavia erecta*	+	+
9	*Calotrophis gigantia*	+	+
10	*Cleome viscosa*	+	+
11	*Croton sparsiflorus*	+	+
12	*Crossandra*	-	-
13	*Coccinia species*	+	+
14	*Commelina benghalensis*	+	+
15	*Corchorus aestuans*	-	+
16	*Corchorus olitorius*	-	-
17	*Corchorus trilocularis*	-	-
18	*Digera arvensis*	-	-
19	*Duranta erecta*	+	+
20	*Euphorbia hirta*	+	+
21	*Euphorbia geniculata*	+	+
22	*Hemidesmus indicus*	-	-
23	*Hyptis suaveolens*	+	+
24	*Indigo species*	+	+
25	*Jatropha curcas*	+	+
26	*Lantana camara*	+	+
27	*Macroptilium*	-	-
28	*Passiflora foetida*	+	+
29	*Pedalium murex*	+	+
30	*Phyllanthus niruri*	-	-
31	*Parthenium hysterophorus*	-	-
32	*Rhynchosia capitata*	-	-
33	*Rhynchosia minima*	-	+
34	*Sonchus arvensis*	+	+
35	*Sida acuta*	+	+
36	*Sida cardifolia*	+	+
37	*Sida rhombifolia*	+	+
38	*Tabernaemontana divaricata*	-	-
39	*Tephrosia purpurea*	+	+
40	*Tridax procumbens*	-	-
41	*Trichodesma indicum*	-	-
42	*Vicia faba*	+	+
43	*Vernonia cinerea*	-	+
44	*Vigna trilobata*	-	-
45	*Ziziphus Spp*	-	-

'+' indicates presences of virus, '-'indicates indicates absence of virus.

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