Plant Pathology Journal

ISSN 1812-5387

science alert

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Plant Pathology Journal 13 (1): 59-64, 2014
ISSN 1812-5387 / DOI: 10.3923/ppj.2014.59.64

Ageratum conyzoides Harbours *Mungbean yellow mosaic India virus*

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Abstract: Yellow mosaic disease caused by *Mungbean yellow mosaic India virus* (MYMIV) is one of the important biotic constraints to mungbean and urdbean production. Information on the weed hosts of MYMIV is meager. *Ageratum conyzoides*, a common weed in and around the agricultural fields throughout the year is often seen with yellow vein symptoms. This prompted us to look into the possibility of *A. conyzoides* acting as an alternate host of MYMIV. The present study deals with the characterization of MYMIV in *A. conyzoides* from four different locations in and around Kanpur. Of the forty plants of *A. conyzoid*es showing yellow vein symptoms subjected to the PCR based detection of viruses causing yellow mosaic disease in pulse crops, 52.5% were found positive with primers specific to MYMIV. None of the samples gave any amplification with primers specific to *Mungbean yellow mosaic virus* and *Horsegram yellow mosaic virus*. Presence of DNA A and DNA B of MYMIV was also confirmed by using specific restriction enzymes to release ~2.7kb DNA fragments from the product of rolling circle amplification. It is evident from the above two detection methods that *A. conyzoides* is a new host of MYMIV. The virus was successfully transmitted by whiteflies from weed to cultivated hosts (mungbean and urdbean) and induced typical yellow mosaic symptoms. Since this is a common weed growing throughout the year, it might be serving as an important source of primary inoculum of this virus for recurrence of yellow mosaic disease in grain legumes in northern India.

Key words: MYMIV, RCA, PCR, ageratum, whitefly

INTRODUCTION

Many researchers have reported weeds to be the alternate hosts and reservoirs of many Geminiviruses that cause diseases in many different crops (Tan *et al*., 1995; Roye *et al*., 1997; Sanz *et al*., 2000; Gilbertson *et al*., 1991; Konate *et al*., 1995). Diverse agro-climatic conditions and rich diversity in plant species in India provide ideal conditions for the perpetuation of plant viruses. According to Malathi and John (2008) yellow mosaic disease of the mungbean and urdbean caused by different begomoviruses is a major production constraint to these crops. Under north Indian conditions mungbean and urdbean, the two important pulse crops cultivated mainly during rainy season (July-September) suffer most due to these viruses. Their cultivation as short duration crop (spring/summer crop) during March-May is also gaining popularity. Based on vector transmission studies, weeds-*Eclipta alba*, *Xanthium strumarium*, *Cosmos bipinnatus* and *Brachiara ramosa* are reported to be the host of *Mungbean yellow mosaic virus* (Nene *et al*., 1971; Nene, 1972).

Ageratum conyzoides, a very common weed in the agricultural fields is often found exhibiting striking yellow vein symptoms. It is known to be the natural host of twelve begomovirus species (Fauquet *et al*., 2008). A geminivirus related to Indian Tomato leaf curl virus (Hallan *et al*., 1998) and recently, *Ageratum enation virus* have been reported to be associated with yellow vein disease of *A. conyzoides* in India (Kumar *et al*., 2011). Pulse crops (grain legumes) like mungbean, urdbean, French bean and pigeonpea are being grown at the Main Research Farm of the Indian Institute of Pulses Research (IIPR), Kanpur since more than four decades. The present study was undertaken with an objective to investigate whether *A. conyzoides* harbours the viruses known to cause yellow mosaic disease in pulse crops.

MATERIALS AND METHODS

Sample collection: Leaves from forty plants (leaf tissues) of *A. conyzoides* L. showing vein yellowing symptoms were collected during November, 2012 from four different locations viz., Main Research Farm and New Research

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Table 1: Details of the samples of *Ageratum conyzoides* tested for the detection of MYMIV

Location	No. of samples collected and subjected to PCR	No. of samples positive with MYMIV specific primers		
		NM1/NM2	MYMIV-AC1F/ MYMIV-AC1R	MYMIV-MPF/ MYMIV-MPR
1	15	9	9	9
2	10	5	5	5
3	10	4	4	4
4	5	3	3	3
Total	40	21	21	21

Location 1: Main Research Farm of IIPR, Kanpur. 2: New Research Farm of IIPR, Kanpur, 3: Research Farm of CSAUAT, Kanpur and 4: Farmers' fields, Fatehpur

Farm of IIPR, Kanpur, Vegetable Research Farm of Chandra Shekhar Azad University of Agriculture and Technology (CSAUAT), Kanpur and farmers' fields, district Fatehpur, Uttar Pradesh, India (Table 1).

Primers used: Primer pairs specific to MYMIV (NM1/NM2), *Mungbean yellow mosaic virus* (MYMV) (MYMV-CPF/MYMV-CPR) and *Horsegram yellow mosaic* virus (HgYMV) (HgYMV-CPF/HgYMV-CPF), the begomo viruses known to cause yellow mosaic disease in different leguminous crops were used in Polymerase Chain Reaction (PCR) as described earlier (Naimuddin *et al*., 2011). Besides these, one more primers pair, MYMIV-AC1F 5'AGT TGA TAT GGA TGT AATAGC3'/ MYMIV-AC1R 5'ACA AAA ACG ACT TCA AATATG CCA A 3' was also used for the detection of MYMIV DNA A.

DNA isolation and amplification of targeted sequence of virus genome: The total DNA was extracted from 40 samples of symptomatic (diseased) and 8 samples of disease free (2 from each location) leaves of *A. conyzoides* by using DNASure Plant Mini Kit (Genetix Biotech Asia Pvt Ltd., New Delhi) as per prescribed protocols. The PCR was performed in a T1 Thermocycler, Biometra® (Goettingen, Germany) programmed for 35 cycles with one step of initial denaturation for 3 min and denaturation for 30 sec at 94°C, 1 min annealing at 54°C for primer pair NM1/NM2, 49°C for primer pair MYMIV-AC1F/MYMIV-AC1R, 60°C for primer pair MYMV-CPF/MYMV-CPR, 57°C for primer pair HgYMV-CPF/HgYMV-CPF, 54°C for primer pair MYMIV-MPF/MYMIV-MPR, 53°C for primer pair HgYMV-MPF/HgYMV-MPR and 54°C for primer pair MYMV-MPF/MYMV-MPR and a 1 min extension at 72°C followed by a one step final extension for 10 min at 72°C. PCR assays were conducted with DreamTaq Green PCR Master Mix (Fermentas) in total reaction mixture volume of 25 μL that contained DNA template (50 ng μL^{-1})-2 μL, primer (25 pmole μL^{-1})-1 μL after each 2×master mix-12.5 μL and dH_2O-8.5 μL. PCR amplification products were resolved in 1% agarose gel electrophoresis in Tris-acetate EDTA (TAE) containing 0.1% ethidium bromide. The gel was examined under a UV trans-illuminator and photographed using a digital camera (Sony DSCW270).

Rolling circle amplification and restriction map: The total DNA from four MYMIV positive samples, one from each location was used to amplify the full length genome of the virus by the RCA method using REPLI-g Mini Kit (QIAGEN GmbH, Hilton). A restriction map of MYMIV genome (EU523045, EU523046) was developed using software RestrictionMapper version 3 (http://www.restrictionmapper.org/) to select the specific restriction enzymes with only one cut site. The RCA products were digested with restriction enzymes specific to DNA A (*Eco*RI, *Eco*RV) and DNA B (*Bgl*I) of MYMIV.

Transmission: Healthy whitefly (*Bemisia tabaci*) colonies maintained on brinjal plants under an insect proof cage were used to transmit the virus from *A. conyzoides* to mungbean and urdbean. Whitefly adults were given 24 h acquisition access feeding on field infected *A. conyzoides* plants that were MYMIV positive in PCR assays. Viruliferous whiteflies were allowed to feed for 24 h on 10 days old healthy mungbean (cv. T44) and urdbean (cv. AKU 9904) plants @ 20/plant. Two sets of ten plants of each mungbean and urdbean were inoculated. After completion of inoculation feeding, whiteflies were killed by spraying plants with insecticide metasystox 25EC @0.05%. Plants were maintained under plastic cages and observed for 30 days for the development of symptoms. Whitefly inoculated plants of mungbean and urdbean were subjected to PCR assays using specific primers pairs for the detection of target virus i.e., MYMIV.

RESULTS

Symptoms: All the diseased samples of *A. conyzoides* collected from different growing sites showed typical yellow vein symptoms. Complete yellowing in newly emerging leaves was also observed (Fig. 1). Samples from different locations could not be differentiated based on symptoms. Ageratum plants with striking yellow vein and leaf curl symptoms appeared to carry geminivirus(es).

Amplification and gel electrophoresis: Results of the PCR tests using total DNA isolated from diseased samples of *A. conyzoides* as template with primers pairs, NM1/NM2 and MYMIV-AC1F/MYMIV-AC1R specifically designed

Fig. 1(a-d): Yellow vein symptoms in *A. conyzoides* collected from different location, (a) Main research farm of IIPR, (b) New research of IIPR, (c) Vegetable research farm of CSAUAT, Kanpur and (d) Farmers' fields, Fatehpur

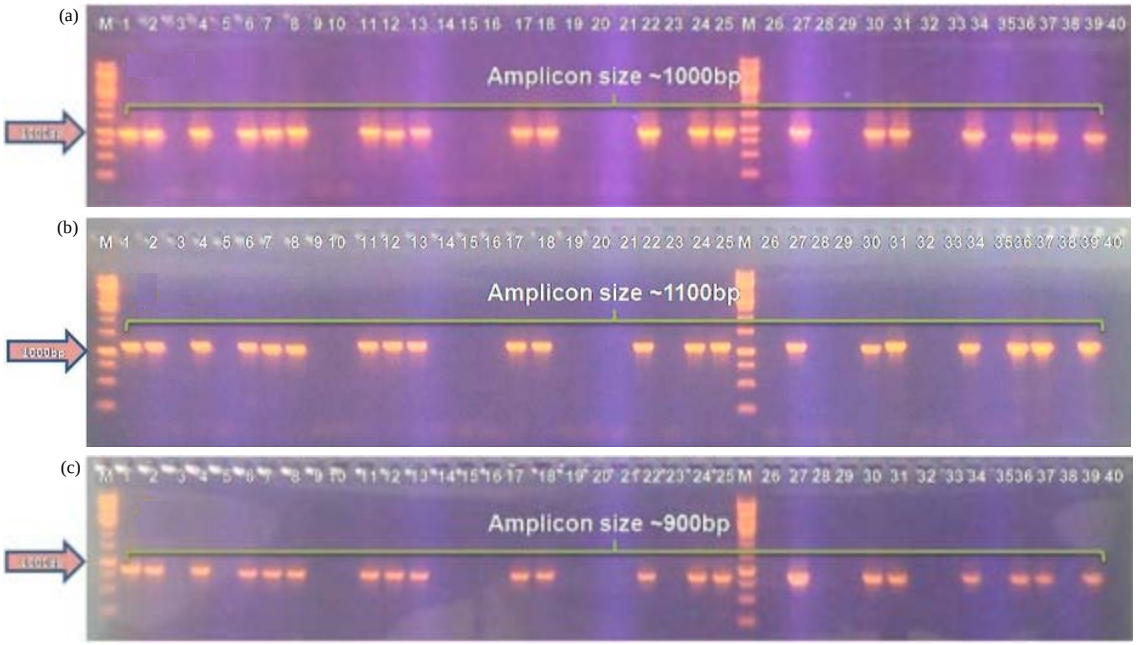

Fig. 2(a-c): Gel electrophoresis of PCR amplified products of MYMIV genome, (a) Segments of DNA A with primer pairs NM1/NM2 and (b) With primer pair MYMIV-AC1F/MYMIV-AC1R, (c) Segment of DNA B with primer pair MYMIV-MPF/MYMIV-MPR. Lane 1-15: Samples from main research farm of IIPR, Lane 16-25: Samples from new research farm of IIPR, Lane 26-35: Samples from vegetable research farm of CSAUAT, Kanpur, Lane 36-40: Samples from farmers' fields of Fatehpur district

to detect a part of DNA A of MYMIV gave amplicons of expected size ~1000 and ~1100 bp, respectively in 52.5% samples (Fig. 2). This indicates that the samples were infected with MYMIV. Since these viruses are bipartite, we also used primers specific to a segment of DNA B of these three viruses (Naimuddin *et al*., 2011). The entire samples positive with MYMIV DNA A also gave positive reaction to MYMIV DNA B in PCR with primers pair,

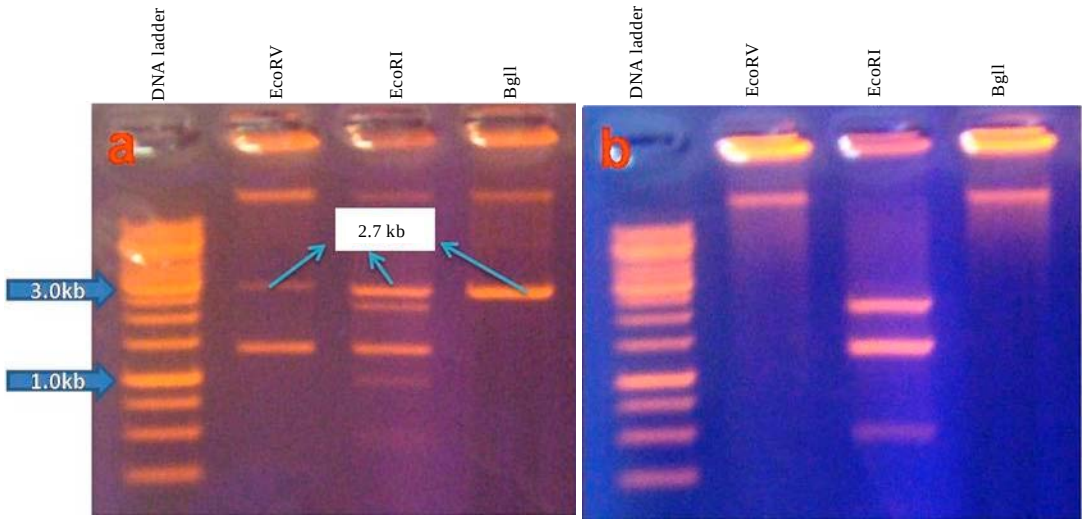

Fig. 3(a-b): Gel electrophoresis of restriction digested RCA products of, (a) MYMIV positive in PCR and (b) MYMIV negative in PCR

MYMIV-MPF/MYMIV-MPR (amplicon size~900 bp). However, none of the 40 samples gave any amplification with primers pairs MYMV-CPF/MYMV-CPR and HgYMV-CPF/HgYMV-CPF specific to DNA A and HgYMV-MPF/HgYMV-MPR and MYMV-MPF/MYMV-MPR specific to DNA B of MYMV and HgYMV, respectively. All the disease free plant samples gave no amplification with any of the primer pairs used.

Rolling circle amplification and restriction map: RCA products of the four samples, positive to MYMIV in PCR tests, digested with restriction enzymes *Eco*RI and *Eco*RV that have only one cut site in DNA A and *Bgl*I that has only one cut site in DNA B of MYMIV produced a band of ~2.7 kb indicating that both the components of MYMIV genome were present in the diseased samples of *A. conyzoides*. Presence of undigested high molecular weight DNA and bands <2.7 kb in the gel, however, indicated possibility of another geminivirus also. Gel electrophoresis of RCA product of one of the samples negative to MYMIV in PCR tests digested with restriction enzymes *Eco*RV and *Bgl*I showed only high molecular weight DNA and no band of ~2.7kb; whereas restriction digestion with *Eco*RI released three DNA fragments with total size of~2.7kb. This indicated that the MYMIV negative sample carried a geminivirus different than MYMIV (Fig. 3).

Transmission: The whiteflies transmitted the virus from field infected *A. conyzoides* to mungbean and urdbean. Symptoms in whitefly inoculated plants started appearing within 10-12 days after inoculation feeding. Percentage of the plants that developed typical yellow mosaic in mungbean and urdbean was 45 and 60, respectively. MYMIV was detected in all the mungbean and urdbean plants that became infected through whitefly transmission but not in the plants that did not develop yellow mosaic symptoms.

DISCUSSION

Virus and virus diseases of crop plants have always got more attention but with the reports of some weed infecting begomoviruses causing diseases in crop plants appeared to have given the impetus to characterize weeds harbouring viruses. In India yellow vein symptoms in this host have been shown to be caused by *Ageratum enation virus* but with enations (Kumar *et al*., 2011). However, the samples collected for present study had only yellow vein symptoms without enations on the leaves. *Ageratum yellow vein virus* (Swanson *et al*., 1993), *Ageratum yellow vein China virus* (Xiong *et al*., 2007), *Ageratum yellow vein Sri Lanka virus* (AF314144), *Malvastrum yellow vein virus* (Jiang and Zhou, 2004), *Sida yellow mosaic China virus* (Xiong and Zhou, 2006), *Pepper yellow leaf curl Indonesia virus* (Shibuya *et al*., 2007) are also reported to cause yellow vein disease on this weed.

Many crop infecting begomoviruses viz., *Papaya leaf curl China virus*-Ageratum,

Pepper yellow leaf curl Indonesia virus, *Tobacco curly shoot virus*, *Tobacco leaf curl Yunnan virus* and *Tomato leaf curl java virus*-B are reported to infect *Ageratum* in different parts of the world (Fauquet *et al*., 2008). *Tomato leaf curl New Delhi virus* (EU688961) and *Tomato yellow leaf curl Tanzania virus* (Kashina *et al*., 2003) have been found infecting *Ageratum* in India and Tanzania, respectively. Present investigation adds *Mungbean yellow mosaic India virus* to the list of crop infecting begomoviruses surviving in *A. conyzoides*. This has significance in understanding the epidemiology of yellow mosaic disease of mungbean and urdbean in northern India.

CONCLUSION

Many begomoviruses have been reported to infect *A. conyzoides*. Present study adds *Mungbean yellow mosaic India virus* to the list of crop infecting begomoviruses surviving in *A. conyzoides*. The findings of the study highlight the need to investigate virus diseases of weeds so as to know the crop infecting viruses being harboured by them. This will help not only in unraveling the epidemiology of many crop infecting begomoviruses but also in devising the management strategies.

ACKNOWLEDGMENTS

We thank Dr. N. Nadarajan, Director, Indian Institute of Pulses Research, Kanpur for providing necessary facilities and constant encouragement.

REFERENCES

Fauquet, C.M., R.W. Briddon, J.K. Brown, E. Moriones, J. Stanley, M. Zerbini and X. Zhou, 2008. Geminivirus strain demarcation and nomenclature. Arch. Virol., 153: 783-821.

Gilbertson, R.L., S.H. Hidayat, R.T. Martinez, S.A. Leong, J.C. Faria, F. Morales and D.P. Maxwell, 1991. Differentiation of bean-infecting geminiviruses by nucleic acid hybridization probes and aspects of bean golden mosaic in Brazil. Plant Dis., 75: 336-342.

Hallan, V., S. Saxena and B.P. Singh, 1998. Short Communication: Ageratum, Croton and Malvastrum harbour geminiviruses: Evidence through PCR amplification. World J. Microbiol. Biotechnol., 14: 931-932.

Jiang, T. and X.P. Zhou, 2004. First report of *Malvastrum yellow vein virus* infecting *Ageratum conyzoides*. New Dis. Rep., 9: 42-42.

Kashina, B.D., R.B.Mabagala and A.A. Mpunami, 2003. First report of *Ageratum conyzoides* L. and *Sida acuta* Burm F. as new weed hosts of *Tomato yellow leaf curl Tanzania virus*. Plant Prot. Sci., 39: 18-22.

Konate, G., N. Barro, D. Fargette, M.M. Swanson and B.D. Harrison, 1995. Occurrence of whitefly-transmitted geminiviruses in crops in Burkina Faso and their serological detection and differentiation. Ann. Applied Biol., 126: 121-129.

Kumar, Y., V. Hallan and A. Zaidi, 2011. First report of *Ageratum enation virus* infecting *Crassocephalum crepidioides* (Benth.) S. Moore and *Ageratum conyzoides* L. in India. J. Gen. Plant Pathol., 77: 214-216.

Malathi, V.G. and P. John, 2008. Geminiviruses Infecting Legumes. In: In: Characterization Diagnosis and Management of Plant Viruses-Vegetables and Pulses Crops, Rao, G.P., K.P. Lava, R.J. Holguin-Pena (Eds.), Studium Press LLC, USA., pp: 97-123.

Naimuddin, M. Akram and A. Pratap, 2011. First report of natural infection of *Mungbean yellow mosaic India virus* in two wild species of *Vigna*. New Dis. Rep., 23: 21-21.

Nene, Y.L., 1972. A Survey of Viral Diseases of Pulse Crops in Uttar Pradesh. G.B. Pant University of Agriculture and Technology, Pantnagar, India, Pages: 191.

Nene, Y.L., J.S. Naresh and N.G. Nair, 1971. Additional hosts of *Mungbean yellow mosaic* virus. Indian Phytopathol., 24: 415-417.

Roye, M.E., W.A. McLaughlin, M.K. Nakhla and D.P. Maxwell, 1997. Genetic diversity among geminiviruses associated with the weed species *Sida* sp., *Macroptilium lathyroides* and *Wissadula amplissima* from Jamaica. Plant Dis., 81: 1251-1258.

Sanz, A.I., A. Fraile, F. Garcia-Arenal, X. Zhou and D.J. Robinson *et al*., 2000. Multiple infection, recombination and genome relationships among begomovirus isolates found in cotton and other plants in Pakistan. J. Gen. Virol., 81: 1839-1849.

Shibuya, Y., J. Sakata, N. Sukamto, T. Kon, P. Sharma and M. Ikegami, 2007. First Report of *Pepper yellow leaf curl Indonesia virus* in *Ageratum conyzoides* in Indonesia. Plant Dis., 91: 1198-1198.

Swanson, M.M., B.D. Harrison and S.M. Wong, 1993. A geminivirus causing vein yellowing of *Ageratum conyzoides* in Singapore. Plant Pathol., 42: 137-139.

Tan, P.H.N., S.M. Wong, M. Wu, I.D. Bedford, K. Saunders and J. Stanley, 1995. Genome organization of ageratum yellow vein virus, a monopartite whitefly-transmitted geminivirus isolated from a common weed. J. Gen. Virol., 76: 2915-2922.

Xiong, Q. and X.P. Zhou, 2006. First report of *Sida yellow mosaic China virus* associated with yellow vein disease of *Ageratum conyzoides* in China. J. Plant Patho., 88: 125-125.

Xiong, Q., F. Sanwei, W. Jianxiang and Z. Xueping, 2007. *Ageratum yellow vein China* virus is a distinct begomovirus species associated with a DNAβ molecule. Virology, 97: 405-411.