

First molecular identification of a begomovirus associated with yellow vein net disease in grain amaranth (*Amaranthus cruentus* L.) in India

S. K. Raj^{A,C}, S. K. Snehi^A, M. S. Khan^A, P. Chandra^B and R. M. Pandey^B

^APlant Molecular Virology, National Botanical Research Institute (NBRI), Lucknow, UP 226001, India.

^BCytogenetics Laboratory, National Botanical Research Institute (NBRI), Lucknow, UP 226001, India.

^CCorresponding author. Email: skraj2@rediffmail.com.

Abstract. The association of a distinct begomovirus with yellow vein net disease in grain amaranths in India was detected by polymerase chain reaction using begomovirus-specific primers. The begomovirus possessed highest identities and closest relationship with *Ageratum enation virus*.

Grain amaranth (*Amaranthus cruentus* L., family Amaranthaceae) is one of the most important subsidiary food crops in the tropical and subtropical highlands of Asia and South Americas (Sauer 1950). The crop is recognised worldwide as an excellent source of high quality and easily digestible protein. A yellow vein net disease affecting 10–15% of plants was observed during the winter of 2007–08 on grain amaranth grown in breeding plots at the National Botanical Research Institute, Lucknow, India. Naturally infected plants exhibited yellow vein net, swelling of veins, reduction of leaf lamina and stunting of the whole plant (Fig. 1). The causal pathogen was suspected to be a begomovirus due to the large population of whitefly (*Bemisia tabaci*) observed in the area and the similarity of the yellow net symptoms to those caused by begomoviruses.

To check for the presence of a begomovirus infection, total DNA was isolated from leaf tissues of five symptomatic and two asymptomatic plants using a published method (Dellaporta *et al.* 1983). The DNA samples were subjected to polymerase chain

reaction (PCR) with a set of primers designed from the coat protein region of begomovirus (GenBank accessions AM180920 and AM180921). As expected, amplicons of ~800 bp were obtained from all five symptomatic but not from the two asymptomatic plant samples. Amplicons obtained from each plant sample were sequenced in both orientations and the consensus sequence was deposited in GenBank database (GenBank accession. EU439259). Basic local alignment search tool (BLAST) search analysis of the sequence data showed highest sequence identity (98%) with *Ageratum enation virus* (AEV) isolates (GenBank accessions AJ437618, DQ343286) and 97% with several isolates of *Tobacco curly shoot virus* (TbCSV, GenBank accessions DQ515969, AJ420318, AJ971266, AJ457986). Genomatix DiAlign analysis with selected begomovirus sequences also showed highest identities of 97–98% at the nucleotide and 100% at the amino acid levels with AEV. The identities of the coat protein region (EU439259) with that of TbCSV isolates were 95–97% and 98–99% at



Fig. 1. Naturally infected *Amaranthus cruentus* showing yellow vein net and stunting of whole plant (left), yellow vein net and swelling of veins on leaf (a close view, right).

Table 1. Percentage identities (nucleotide and amino acid) in coat protein gene of the virus isolate from *Amaranthus cruentus* (EU439259) various begomovirus based on the Genomatix DiAlign program

AEV, *Ageratum enation virus*; TbCSV, *Tobacco curly shoot virus*; ToLCV, *Tomato leaf curl virus*; AYVV, *Ageratum yellow vein virus*; ToLCBaV, *Tomato leaf curl Bangladesh virus*; ChLCPaV, *Chilli leaf curl Pakistan virus*; PLCV, *Papaya leaf curl virus*

GeneBank accession	Virus isolate	Natural hosts	Location/Country	Percentage identity ^A	
				Nucleotide	Amino acid
AJ437618	AEV	<i>Ageratum conyzoides</i>	Nepal	98 (0.957)	100 (1.000)
DQ343286	AEV	<i>Ageratum conyzoides</i>	India	97 (0.944)	100 (1.000)
AJ971266	TbCSV	<i>Ageratum conyzoides</i>	China	97 (0.924)	99 (0.985)
AJ420318	TbCSV	<i>Nicotiana tabacum</i>	China	97 (0.923)	99 (0.985)
DQ515969	TbCSV	<i>Dimorphotheca sinuata</i>	India	97 (0.930)	98 (0.973)
AY887174	TbCSV	<i>Calendula officinalis</i>	India	97 (0.922)	99 (0.985)
AJ457986	TbCSV	<i>Lycopersicon esculentum</i>	China	97 (0.913)	98 (0.977)
AF240675	TbCSV	<i>Nicotiana tabacum</i>	China	95 (0.861)	98 (0.984)
AY690431	ToLCV	<i>Lycopersicon esculentum</i>	India	95 (0.885)	98 (0.976)
AF188481	ToLCBaV	<i>Lycopersicon esculentum</i>	Bangladesh	94 (0.822)	98 (0.981)
AJ810825	AYVV	<i>Ageratum conyzoides</i>	Pakistan	94 (0.868)	94 (0.931)
DQ116879	ChLCPaV	<i>Capsicum annuum</i>	Pakistan	93 (0.810)	96 (0.964)
DQ376036	PLCV	<i>Carica papaya</i>	India	92 (0.822)	98 (0.973)

^ASimilarity value given in bracket = 1.000 marks only the two most similar sequences.

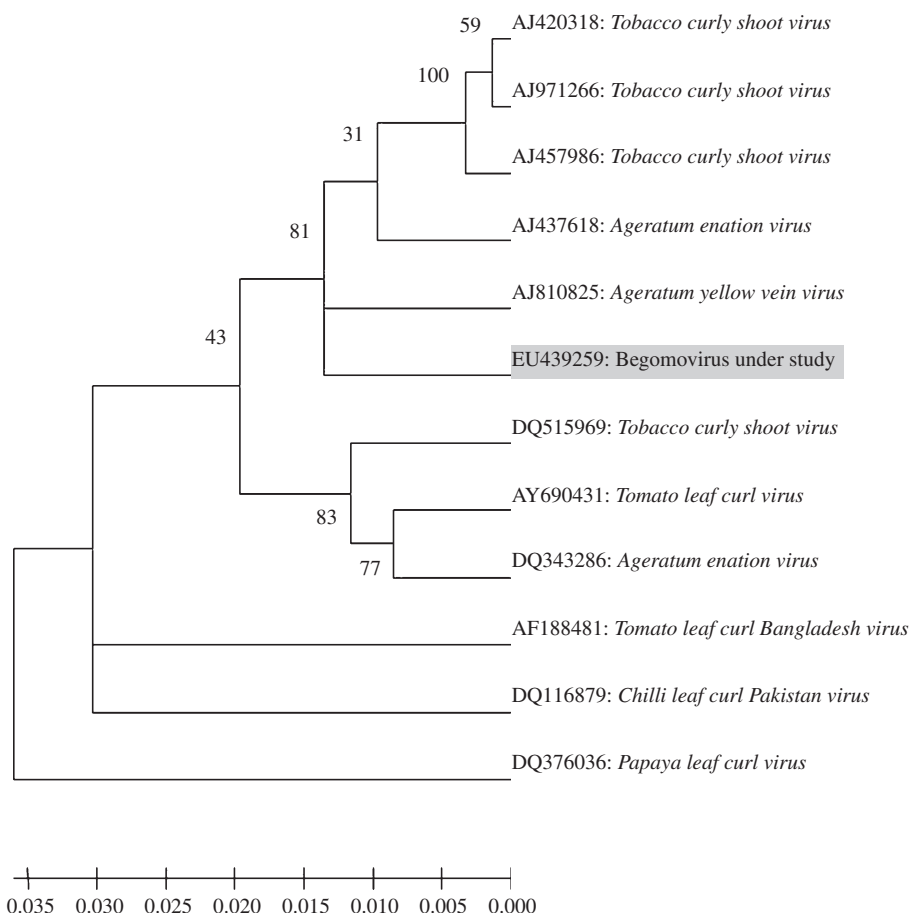


Fig. 2. Phylogenetic relationships of the virus isolate (GenBank accession EU439259) at nucleotide level with *Ageratum yellow vein virus*, *Ageratum enation virus*, *Tobacco curly shoot virus* and other begomovirus isolates reported from India and abroad. The phylogenetic tree was generated in MEGA 4.0 version using 100 bootstrap values.

nucleotide and amino acid levels, respectively (Table 1). *Ageratum yellow vein virus* (AYVV) showed 94% identities at nucleotide and the amino acid levels. Phylogenetic analysis of the selected begomovirus isolates using molecular evolutionary genetics analysis (MEGA) 4.0 tool (Tamura *et al.* 2007) showed the closest relationships with AYVV, AEV and TbCSV isolates and more distant relationships with other begomovirus isolates (Fig. 2). The closest relative of the begomovirus under study could not be determined using the phylogentic tree as the distinction between AEV, AYVV and TbCSV was not well supported (i.e. a bootstrap value of only 31). The virus isolate is, therefore, proposed to be a begomovirus closely related to AEV based on the highest (98%) sequence identity, and in accordance with the latest report of International Committee on Taxonomy of Viruses (Fauquet *et al.* 2008).

Tomato yellow leaf curl virus has been detected by nucleic acid tissue blot hybridisation in *Amaranthus* sp. (Abou-Jawdah *et al.* 1999). *Amaranthus spinosus* and *A. viridis* weeds are reported as hosts of begomoviruses (Arnaud *et al.* 2007). Several species of *Amaranthus* have also been found to be the natural hosts of other groups of viruses (Horvath 1991; Raj *et al.* 1997). However, natural occurrence of a begomovirus (closely related to *Ageratum enation virus*) in *A. cruentus* is a first report from India. *Amaranthus cruentus* may be considered as an alternate host of AEV which might be playing a key role in its dissemination to other important crop species.

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References

- Abou-Jawdah Y, Maalouf R, Shebaro W, Soubra K (1999) Comparison of the reaction of tomato lines to infection by tomato yellow leaf curl begomovirus in Lebanon. *Plant Pathology* **48**, 727–734. doi: 10.1046/j.1365-3059.1999.00403.x
- Arnaud LSEP, Santos CDG, Lima JAA (2007) Predominance of begomovirus on tomato in chapada da Ibiapaba, Ceará, and its natural detection on weeds plants. *Fitopatologia Brasileira* **32**, 241–246. doi: 10.1590/S0100-41582007000300009
- Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA miniprep: Version III. *Plant Molecular Biology Reporter* **1**, 19–21. doi: 10.1007/BF02712670
- Fauquet CM, Briddon RW, Brown JK, Moriones E, Stanley J, Zerbini M, Zhou X (2008) Geminivirus strain demarcation and nomenclature. *Archives of Virology* **153**, 783–821. doi: 10.1007/s00705-008-0037-6
- Horvath J (1991) *Amaranthus* species (Family Amaranthaceae) as host of plant viruses: a review. *Acta Phytopathologica et Entomologica Hungarica* **26**, 385–422.
- Raj SK, Aminuddin, Singh BP, Pal M (1997) Characterization of a cucumber mosaic virus isolate causing leaf crinkle and severe mosaic of *Amaranthus* in India. *Canadian Journal of Plant Pathology* **19**, 97–100.
- Sauer JD (1950) The grain amaranths: A survey of their History and Classification. *Annals of the Missouri Botanical Garden* **37**, 561–632. doi: 10.2307/2394403
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596–1599. doi: 10.1093/molbev/msm092

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