First report of a begomovirus infecting the ornamental plant *Vinca minor* L.

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Abstract.   *Vinca minor* L., an ornamental plant with symptoms typical of a begomovirus was found in the vicinity of the School of Biological Sciences, Lahore, Pakistan. Symptomatic and apparently healthy leaf samples were collected and subjected to DNA extraction and polymerase chain reaction, using universal primers conserved for the coat protein region of begomoviruses. An expected size product of ~0.78 kb was amplified from symptomatic leaf samples while healthy plant samples gave no amplification. The product was cloned and sequenced in its entirety. Basic Local Alignment Search Tool analysis showed 93% nucleotide sequence identity with *Pedilanthus leaf curl virus* originating from Pakistan. Our results indicate that this is the first report of a begomovirus infecting *V. minor* in Pakistan.

Vinca minor L. (common name Periwinkle, Sadabahar; family Apocynaceae) is a vine sub-shrub plant commonly grown as an ornamental plant in orchards and house lawns in Pakistan. During a survey for begomoviruses in ornamental plants, symptoms typical of begomoviruses, including yellowing, leaf curl and distorted leaves, were observed on the *V. minor* plants (Fig. 1). The infection was estimated to affect 30–40% of plants surveyed, based on visual observation of symptoms.
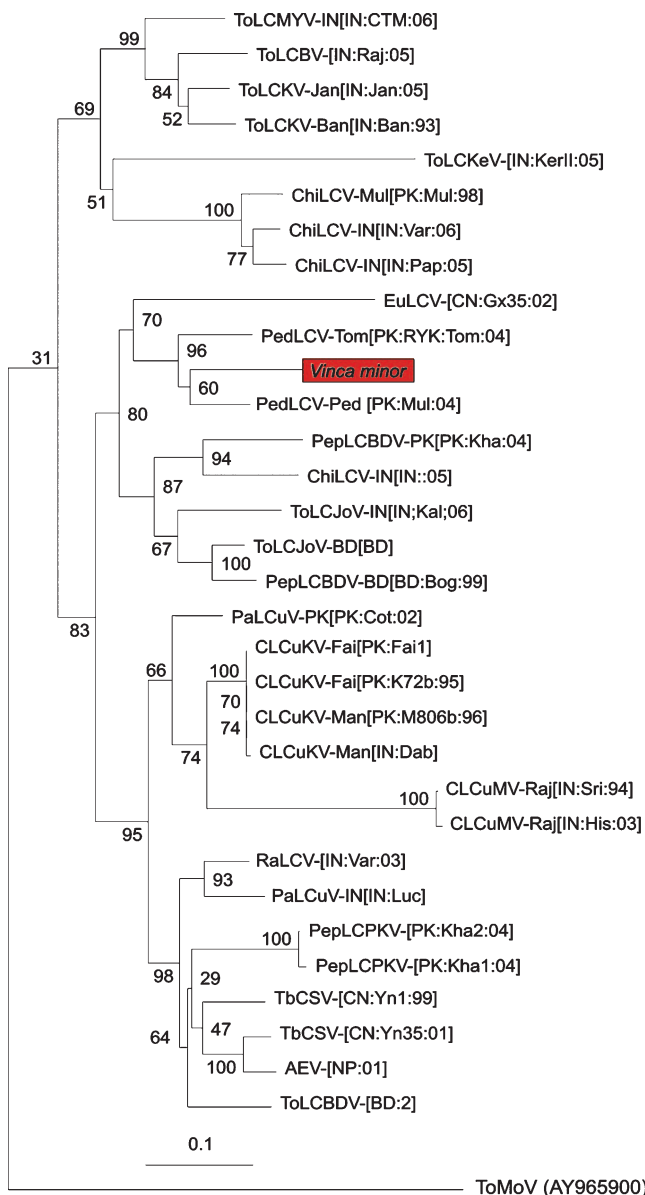
Total DNA from two symptomatic and one healthy leaf sample of *V. minor* was extracted by the cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). The presence

of a begomovirus was confirmed by polymerase chain reaction (PCR) amplification using a degenerate primer pair, CPF 5′-ATG (C/A/T)(G/C)(G/C/A)AAGCG(A/T)(C/A)C(A/C)G(G/C)(A/C) GATAT-3′ and CPR 5′-TTAATT(T/G/C)(C/G/A)(A/T/C)(A/T/G) A(C/T)(A/T/C)(G/C)(C/A/T)(A/G)TCATA(G/A)AA(A/G)TA-3′, designed from published sequences of begomoviruses to conserved regions of the coat protein genes from the Old World (Haider *et al*. 2006). An expected size product of ~0.78 kb was amplified from symptomatic samples but not from the healthy plant sample. The product was cloned from one of the samples into a T/A cloning vector, pTZ57R/T (Fermentas Inc.), according to the manufacturer's instructions and sequenced in its entirety with no ambiguity remaining. Sequences were determined by dideoxynucleotide chain-termination sequencing using GenomeLab Dye Terminator Cycle Sequencing kits (Beckman Coulter) on a Beckman Coulter automated sequencer (CEQ 8000). Sequences were assembled and analysed using DNASTAR (Lasergene). Multiple sequence alignments and a phylogenetic tree were produced using ClustalX. A phylogenetic tree was manipulated and printed using Treeview (Page 1996) and submitted to the EMBL database under the accession number AM292303. Comparison of the sequence isolated from *V. minor* with sequences available in the databases showed a maximum of 93% nucleotide sequence identity with a previously described begomovirus from Pakistan, *Pedilanthus leaf curl virus*-Pedilanthus (Pakistan:2004, abbreviated as PedLCV-Ped [PK:Mul:04], accession number AM712436; Tahir *et al*. 2008), followed by 92% nucleotide sequence identity with PedLCV-Tomato ([PK:RYK:Tom:04], accession number DQ116884).

According to recent descriptions on demarcation and nomenclature of Geminivirus strains, Fauquet *et al*. (2008) have outlined viruses with nucleotide identities <93% across the whole genome as strains and >94% as variants of that strain in

Fig. 1.   *Vinca minor* with distorted, curled and yellowed leaf symptoms (right) and a healthy *V. minor* plant (left).

Fig. 2. Phylogenetic dendrogram based upon alignments of the complete coat protein sequences of begomoviruses. The phylogentic dendogram was built using the most similar begomovirus coat protein sequences with the coat protein sequence of the begomovirus isolated from Vinca as identified in a BLAST search. The virus sequences used are *Tomato leaf curl Malaysia virus* (ToLCMYV-IN[IN:CTM:06], DQ629102), *Tomato leaf curl Rajasthan virus* (ToLCBV-[IN:Raj:05], DQ339117), *Tomato leaf curl Karnataka virus* (ToLCKV-Jan[IN:Jan:05], Y754812, ToLCKV-Ban[IN:Ban:93], U38239), *Tomato leaf curl Kerala virus* (ToLCKeV-[IN:KerII:05], DQ852623), *Chilli leaf curl virus* (ChiLCV-Mul[PK:Mul:98], AF336806, ChiLCV-IN[IN:Var:06], EF190217, ChiLCV-IN[IN:Pap:05], DQ989326, ChiLCV-IN [IN:05], DQ673859), *Euphorbia leaf curl virus* (EuLCV-[CN:Gx35:02], AJ558121), *Pedilanthus leaf curl virus* (PedLCV-[PK:04], AM712436, PedLCV-[PK:RYK:Tom:05], AB116884), *Pepper leaf curl Bangladesh virus* (PepLCBDV-PK[PK:Kha:04], DQ116881, PepLCBDV-BD[BD: Bog:99], AF314531), *Tomato leaf curl Joydebpur virus* (ToLCJoV-BD [BD], AJ875159, ToLCJoV-IN[IN:Kal:06], EF194765), *Papaya leaf curl virus* (PaLCuV-PK[PK:Cot:02], AJ436992, PaLCuV-IN[IN:Luc], Y15934), *Cotton leaf curl Kokhran virus* (CLCuKV-Fai[PK:Fai1], AJ496286, CLCuKV-Fai[PK:K72b:95], AJ002448, CLCuKV-Man[PK:M806b:96] AJ002449, CLCuKV-Man[IN:Dab], AY456683), *Cotton leaf curl Multan virus* (CLCuMV-Raj[IN:Sri:94], AF363011, CLCuMV-Raj[IN:His:03], AY795607), *Radish leaf curl virus* (RaLCV-[IN:Var:03], EF175733), *Pepper leaf curl Pakistan virus* (PepLCPKV-[PK:Kha1:04], DQ116878, PepLCPKV-[PK:Kha2 : 04], DQ116879), *Tobacco curly shoot virus* (TbCSV-[CN:Yn1:99], AF240675, TbCSV-[CN:Yn35:01], AJ420318), *Ageratum enation virus* (AEV-[NP:01], AJ437618) and *Tomato leaf curl Bangladesh virus* (ToLCBDV-[BD:2], AF188481). The tree was arbitrarily rooted on the sequence of the DNA A component of *Tomato mottle virus* (ToMoV-[PR:04], AY965900), a distantly-related begomovirus originating from the New World. The numbers at nodes indicate bootstrap confidence values (100 replicates). The position of the sequence originating from the begomovirus isolated from *V. minor* is indicated with a red box. Abbreviations used: IN, India; CTM, Cherry Tomato; Raj, Rajasthan; Jan, Janti; Ban, Banglore; Ker, Kerala; PK, Pakistan; Mul, Multan; Var, Varanasi; Pap, Papaya; Gx, Guangxi; CN, China; Tom,Tomato; RYK, Rahim Yar Khan; Kha, Khanewal; BD, Bangladesh; Kal, Kalyani; Bog, Bogra; Cot, Cotton; Fai, Faisalabad; K, Kokhran; Man, Manisal; Dab, Dabawali; Sri, Sriganganagar; His, Hisar; Luc, Lucknow; Yn, Yunnan; NP, Nepal; PR, Puerto Rico.

that species, although biological data such as host range and symptom expression can also be used to define a strain. Since the virus isolate from *V. minor* showed highest identity (93%) with PedLCV-Ped [PK:Mul:04] across 0.78 kb of the coat protein gene, it is likely to be a strain of PedLCV-Ped [PK:Mul:04]. Conclusive nomenclature of the begomovirus isolated from *V. minor* will be based on its complete genome nucleotide sequence.

A phylogenetic analysis of the complete coat protein nucleotide sequence isolated from *V. minor* with the most similar sequences in the databases (determined by Basic Local Alignment Search Tool analysis) is shown in Fig. 2. This confirms close similarity with PedLCV. This is the first report of *V. minor* being infected with a begomovirus. An evergreen plant infected with a begomovirus is a serious threat to other economically important crops as it acts as a permanent reservoir of virus. Future studies will be focussed on the amplification of the complete genome of the virus, its phylogeny, and a detailed survey of its distribution and host range in distinct locations of the country.

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