Complete sequence of RNA3 of Cucumber mosaic virus isolates infecting *Gerbera jamesonii* suggests its grouping under IB subgroup

K. K. Gautum · R. Raj · S. Kumar ·
S. K. Raj · R. K. Roy · R. Katiyar

Received: 12 March 2014 / Accepted: 30 April 2014 / Published online: 24 May 2014
© Indian Virological Society 2014

Abstract The complete RNA3 genome of *Cucumber mosaic virus* (CMV) was amplified by RT-PCR from three infected gerbera (*Gerbera jamesonii*) leaf samples exhibiting severe chlorotic mosaic and flower deformation symptoms. The amplicons obtained were cloned sequenced and deposited in GenBank under the accessions JN692495, JX913531 (from cv. Zingaro) and JX888093 (from cv. Silvester). These sequences shared 98–99 % identities to each other and with a strain of CMV-Banana reported from India, and 90–95 % identities with various strains of CMV reported worldwide. Phylogenetic analysis revealed their closest affinity with CMV-Banana strain, and close relationships with several other strains of CMV of subgroup IB. This study provides evidence of subgroup IB CMV causing severe chlorosis and flower deformation in two cultivars (Zingaro and Silvester) of *G. jamesonii* in India.

Keywords *Gerbera* · RNA3 genome · *Cucumber mosaic virus* · IB subgroup isolates

Electronic supplementary material The online version of this article (doi:10.1007/s13337-014-0216-x) contains supplementary material, which is available to authorized users.

K. K. Gautum · R. Raj · S. Kumar · S. K. Raj (✉)
Plant Molecular Virology Laboratory, CSIR-National Botanical
Research Institute, Lucknow 226001, India
e-mail: skraj2@rediffmail.com

R. K. Roy
Botanic Gardens, CSIR-National Botanical Research Institute,
Lucknow 226001, India

R. Katiyar
Department of Botany, University of Lucknow,
Lucknow 226007, India

Gerbera (*Gerbera jamesonii*, family *Asteraceae*) is an exotic flowering species of high economic importance in floriculture industry and cultivated commercially for cut-flower sticks, besides popularly grown for decorative gardens and pot plants [8]. Due to its ability to withstand long transportation, gerbera has been considered suitable for export as well as domestic markets of India [1]. For this reason, growers in Karnataka, Maharashtra, North-Eastern and Uttar Pradesh states have accepted its commercial cultivation in India. *Gerbera* cultivation has major constraint of natural infection of several viruses which deteriorate the commercial quality of flowers, thereby lowering the economy of growers and country too. Literature revealed the occurrence of *Chrysanthemum stem necrosis virus*, *Impatiens necrotic spot virus* [3]; *Tomato black ring virus* [10]; *Tomato spotted wilt virus* [2]; *Tobacco mosaic virus* [11]; *Tobacco rattle virus* [5]; and *Cucumber mosaic virus* (CMV) [4, 10, 11] on gerbera plants causing variety of disease symptoms and tremendous flower yield reduction. In present study, we report the complete RNA3 genome sequences of three new isolates of CMV of subgroup IB associated with severe chlorotic mosaic and flower deformation disease in two cultivars of *G. jamesonii* based on sequence analyses of RNA3 genome.

During the survey in Nov–Dec months of 2011, chlorotic mosaic disease was observed on a number of gerbera plants growing in a polyhouse (Fig. 1a) at CSIR-NBRI, Lucknow. The disease incidence was 16.27 % (70/430) in cv. Zingaro followed by 11.57 % (50/432) in cv. Silvester. The diseased plants exhibited severe chlorotic mosaic, floret and flower deformations along with color breaking (Fig. 1b, c) symptoms as compared to healthy one (Fig. 1d), suspecting to be a viral disease.

The transmission of causal virus was attempted using the leaf sap of naturally infected gerbera plants of cultivars

Fig. 1 Naturally infected gerbera plants exhibiting chlorotic mosaic symptom in polyhouse conditions (a), a close view of infected leaf showing severe chlorotic mosaic symptom (b) and severe flower deformation (c) as compared to healthy one (d). Phylogenetic analysis of RNA3 genome sequences of gerbera virus isolates under study (JN692495, JX913531 and JX888093) with known CMV strains of subgroup IA, IB and II (e) showing close relationships with Indian strains of CMV of subgroup IB. Phylogram was generated using MEGA v5.1 tool with 1,000 bootstrap replicates and PSV and TAV were used as reference out group sequences of cucumovirus genus for the rooting of tree. Asterisks denotes for exceptions

(cvs.) Zingaro and Silvester, separately on recipient host seedling: *Chenopodium amaranticolor*, *C. album*, *Spinacia oleracea*, *Cucumis sativus*, *Capsicum annuum*, *Cucumis sativus*, *Petunia hybrida*, *Nicotiana glutinosa*, *N. tabaccum* cv. White Burley, *N. rustica* and *G. jamesonii*. During sap transmission tests, the virus was successfully transmitted from naturally infected gerbera to healthy gerbera seedlings which developed similar chlorotic mosaic symptoms at 40–45 dpi, suggesting the Koch's postulates. The inoculations of sap taken from cultivars Zingaro and Silvester also induced more or less similar local and necrotic lesions on *C. amaranticolor* and *C. album*; chlorotic local lesions on *S. oleracea* and *C. sativus* at 7 dpi. While systemic mosaic symptoms were developed on *C. sativus*, *C. annuum*, *P.*

hybrida, *N. glutinosa*, *N. tabaccum* cv. White Burley and *N. rustica* at 30–35 dpi.

For molecular detection of the virus, the total RNA was extracted from 100 mg leaf tissue of symptomatic plants of gerbera cv. Zingaro and Silvester using TRIzol (Sigma, USA) and reverse transcription-polymerase chain reaction (RT-PCR) was performed using CMV-CP gene specific primers as described earlier [6]. Electrophoresis of RT-PCR products resulted in amplification of expected size ~ 650 bp bands in naturally infected gerbera samples of cv. Zingaro and Silverster, similar to as in CMV-Banana strain taken as positive control, confirming the presence of CMV.

Further, the complete RNA3 genome was amplified from three infected representative samples of two cv.

Zingaro and Silvester using CMV-RNA3 specific primers as described earlier [6]. Three amplicons of ~2.2 kb obtained from Zingaro (two) and Silvester (one) were cloned into pGEM-T vector (Promega Corp., Madison, USA) and three positive clones for each amplicon were got sequenced. The sequence data obtained were analyzed, assembled and consensus sequence for RNA3 genome determined was deposited to GenBank database under accession numbers: JN692495 (Zing.1) and JX913531 (Zing.2) and JX888093 (Silves.1). The analysis of sequence data revealed the presence of full length RNA3 of 2219 nucleotides consisted of two open reading frames (ORFs): movement protein gene of 840 nucleotides (ATG and TAG at 123 and 962 nucleotide positions, respectively) putatively translating 229 amino acids and coat protein (CP) gene of 657 nucleotides (ATG and TAG at 1261 and 1917 nucleotide positions, respectively) translating 218 amino acid residues. Both the ORFs were separated by 300 nucleotide long intergenic region and flanked by 5'untranslated region (UTR) and 3'UTR of 122 and 302 nucleotides, respectively.

To observe the sequence identity of virus isolates under study with existing sequences available in GenBank, the obtained sequence data was first analyzed by BLASTn (<http://www.ncbi.nlm.nih.gov/Blast.cgi>). During BLASTn, RNA3 genome of three isolates: JN692495 (Zing.1) and JX913531 (Zing.2) and JX888093 (Silves.1) shared 98–99 % nucleotide sequence identities to each other whereas 97–99 % identities with CMV-Banana (EF178298) and -Eggplant (HQ343232) strains of subgroup IB, reported from India. The isolates under study also shared 92–95 % sequence identity with other CMV strains (JN642676, EF593024, EF593025, EF153733, EF593023, EF593026, JN054635, AB046951, EF216867, DQ006805, FJ268746, EF216865, DQ412732, HQ283393, Y16926, EF153734, D28780 and AM183116 of subgroup IB reported worldwide. The specific sequence identities among the selected subgroup IA, IB and II group members were also determined by *Genomatix DiAlign* 2.1 program [7] and the data obtained was summarized in supplementary Table 1. During alignment analysis, RNA3 genomes under study shared 97–99 % sequence identities to each other which may be well correlated with the results of BLASTn. The isolates Zing.1 and Zing.2 showed 97 % identity with Banana isolate of CMV of subgroup IB reported from India while Silves.1 isolate showed 99 % identity. They also showed 89–94 % identities with various other isolates of CMV subgroup IB reported worldwide. However, identities were 84–86 % with isolates of CMV subgroup IA and 62–63 % with isolates of CMV subgroup II (Supplementary Table 1).

To infer the phylogenetic relationships of virus isolates under study with selected CMV strains of subgroup IA, IB

and II (Supplementary Table 1), the phylogenetic analysis was performed using MEGA v5.1 program [9]. The phylogram was generated with 1,000 bootstrap replicates and *Peanut stunt virus* and *Tomato aspermy virus* were used as reference out group sequences of *cucumovirus* genus for the rooting of tree. Phylogenetic analysis clearly grouped the subgroup IA, IB and II members to their respective clusters. The virus isolates under study clustered together and showed closest relationship with CMV-Banana, -Eggplant and -Petunia strains reported from India (Fig. 1e). Though the virus isolates under study clustered together, however, Zing.1 and Zing.2 isolates were found closest as compared to Silves-1 isolate. Based on highest 97–99 % sequence identities and closed phylogenetic relationships with various CMV strains of subgroup IB, the virus isolates under study were identified as new isolates of CMV of subgroup IB.

The natural occurrence of CMV on gerbera is reported earlier from Australia [4], India [10] and China [11]. The only report from India described the evidence of CMV by ELISA and analysis of a small sequence amplified by RT-PCR from gerbera exhibiting colour break symptoms on petals, asymmetrical ray florets, and deformed flowers [10]. However, the complete identification and proper sub-grouping (IA or IB) of the CMV isolate was lacking. In present study, we report the complete RNA3 genome sequences of three new isolates of CMV of subgroup IB associated with severe chlorotic mosaic and flower deformation disease in two cultivars of *G. jamesonii*. Since the CMV-Gerbera strains cause severe flower deformations and deteriorate greatly the trade value of gerbera flowers, further virus management seems to be very essential. The information on complete RNA3 genome sequences will help in development of sensitive, reliable diagnostics, and further genetic engineering based management strategies to circumvent the CMV infection in gerbera.

Acknowledgments Authors are thankful to the Director, CSIR-National Botanical Research Institute, Lucknow for facilities, Indian Council of Medical Research, New Delhi for fellowship to K. K. Gautam and Council of Scientific & Industrial Research, New Delhi for funding as PMSI project number BSC-117.

References

1. Aswath C, Rao TM. Breeding of gerbera (*Gerbera jamesonii* Bolus ex. Hooker F.) lines suitable for open field cultivation. *J Ornament Hort.* 2006;9:243–7.
2. Chatzivassiliou EK, Livieratos I, Jenser G, Katis NI. Ornamental plants and thrips populations associated with *Tomato spotted wilt virus* in Greece. *Phytoparasitica.* 2000;28:257–64.
3. Elliott DR, Lebas BSM, Ochoa-Corona FM, Tang J, Alexander BJR. Investigation of impatiens necrotic spot virus outbreaks in New Zealand. *Australas Plant Pathol.* 2009;38:490–5.

4. Finlay JR. Cucumber mosaic virus in gerbera. *Australas Plant Pathol.* 1975;4:14.
5. Hakkaart FA. A virus disease of *Gerbera jamesonii*. *Eur J Plant Pathol.* 1968;74:28–9.
6. Kumar S. Ph.D. Thesis, Molecular characterization of Cucumoviruses causing severe mosaic and ringspot diseases in chrysanthemum and development of their management strategies. University of Lucknow, Lucknow; 2009.
7. Morgenstern B. DIALIGN 2: improvement of the segment- to-segment approach to multiple sequence alignment. *Bioinformatics.* 1999;15:211–8.
8. Parthasarathy VA, Nagaraju V. In vitro propagation in *Gerbera jamesonii* Bolus. *Indian J Hortic.* 1999;56:82–5.
9. Tamura K, Dudley J, Nei M, Kumar S. MEGA5.1: molecular evolutionary genetics analysis (MEGA) software version 5.1. *Mol Biol Evol.* 2011;24:1596–9.
10. Verma N, Singh AK, Singh K, Raikhy LS, Hallan V, Ram R, Zaidi AA. Occurrence of cucumber mosaic virus in *Gerbera jamesonii* in India. *Plant Dis.* 2004;88:1161.
11. Zhang L, Yang C, Yu C, Yuan W, Ma Q. Rapid detection of 3 viruses in *Gerbera jamesonii* Bolus and its virus-free by tissue culture. *Acta Phytophylacica Sin.* 2009;36:239.