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SHORT COMMUNICATIONS

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Identification of Dahlia Mosaic Virus by Molecular Methods

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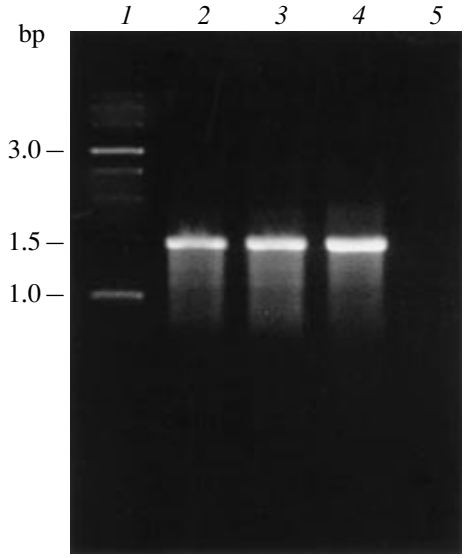
In Russia, the dahlia mosaic virus (DMV), classified as a caulimovirus, was first detected during the study of leaf ultrastructure in plants from the collection of the Main Botanical Garden of the Russian Academy of Sciences in the 1980s [1]. In the southern Far East, DMV was detected in dahlia in the late 1990s. Its taxonomic position was determined by comparing certain biological features of the isolate with those described in the literature [2, 3]. This publication deals with the results of further research on the virus and the development of a method for its identification by modern molecular methods.

The dahlia specimens with virus-like symptoms used in the study were collected in the Khabarovsk Region and Primorye in 2004. Total DNA was isolated from 50 mg of leaf tissue with a DNA isolation kit (Medigen Laboratory, Russia) and dissolved in 50 μl of water. The DNA of DMV in the samples was assayed by polymerase chain reaction (PCR) in a T3 thermocycler (Biometra, Germany). The D1F (forward) and D1R (reverse) primers, shown in the table, border a 1428-bp stretch of the DMV gene IV, which codes for a capsid protein. The primers were designed with reference to the only nucleotide sequence of the corresponding DMV genome fragment stored in the EMBL database (accession no. AY291588), using the Gene Runner program (Hastings Software, USA). The reaction was carried out with a kit including *Taq* DNA polymerase (SibEnzyme, Russia). The reaction volume (25 μl) contained 4 μl of a DNA sample and 0.5 μmol of each primer. The PCR program was conducted as follows: a predenaturation step for 180 s at 95°C followed by 30 cycles: denaturation at 93°C for 30 s, annealing at 61°C for 30 s, and elongation at 72°C for 80 s. The method was tested on the DMV-8 isolate obtained in Primorye in 2004.

The electrophoretic pattern of PCR products in 1% agarose gel is shown in the figure. The reaction under optimized conditions gave rise to a single DNA fragment 1440 bp in length, which corresponded to the predicted value.

The specificity of the D1F–D1R primer pair was tested in PCR with DNA from uninfected plants. The characteristic fragment was not detected there. However, no positive control DMV sample was available, and further experiments were aimed at verification of the species-specific nature of the fragments obtained in the first PCR round. Bands with PCR products were cut from the gel, purified with a GenElute PCR Clean-Up kit (Sigma), and used as a template for reamplification with internal primers D2F–D2R (table). The sample volumes were 10 μl. The sample contained 0.3 μmol of each primer and 1 μl of the purified PCR



Electrophoresis of products obtained by PCR with the D1F–D1R primers: (*1*) molecular weight marker DNA Ladder 1 Kb (SibEnzyme), (*2–4*) lanes corresponding to the specific PCR product of DNA from plants infected with DMV, and (*5*) control amplification of DNA from an uninfected plant.

Primers used in the study

Primer	Sequence (5' ⟶ 3')	Location*
D1F	ATG–GCC–TCC–AGT–ATG–AAA–GAA–A	1–22
D1R	CTG–TTC–CTG–ATG–ATT–CAT–CAT–C	1450–1471
D2F	AAA–GAA–CAT–CAA–CTT–AGT–AGC–C	843–865
D2R	GCT–TGG–GCC–TAG–TAT–ATT–TC	1146–1165

* Location of primer sequences in DMV gene IV (sequence AY291588).

product obtained with the external primers and diluted 1:1000. The PCR program involved predenaturation at 94°C for 120 s followed by 25 cycles: denaturation at 93°C for 20 s, annealing at 50°C for 15 s, and elongation at 72°C for 20 s. The consensus sequence was constructed with the LaserGene program (DNA-Star, USA) and compared with sequences stored in GenBank with the BLAST program (http://www.ncbi.nlm.nih.gov). A 323-bp nucleotide sequence (EMBL accession no. AY971810) is highly similar (>97%) to the corresponding fragment of the AY291588 sequence, which confirms specific amplification in the first PCR round.

The DMV genome was identified with the proposed primer pair D1F–D1R. Viral DNA was detected in 12 out of 37 samples (32.4%). All positive samples were taken from plants grown in Primorye. It is likely that the virus is widespread in this region.

Thus, additional evidence for the presence of dahlia mosaic virus in Russia, based on amplification and sequencing of gene IV, was obtained in this work. The proposed PCR primers and conditions can be applied to virus detection, along with conventional virological approaches.

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