Dahlia Mosaic-Associated Caulimoviruses in Dahlia in Lithuania

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In recent years, field floriculture development in Lithuania has been given increased attention particularly for the benefit of small family farms that produce seedlings of perennial ornaments for the domestic market and for export to the neighboring countries. Like other segments of agriculture, this expanding sector is threatened by plant diseases. Dahlia (Dahlia variabilis) is one of the important flower crops in Lithuania with ornamental value and economic potential. Several new cultivars of dahlia were recently developed in Lithuania (1). Recent surveys, based on biological and serological assays, revealed that dahlia was infected with several viruses including Dahlia mosaic virus (DMV), Tomato ring spot virus, and Tomato spotted wilt virus (3). To better understand the incidence and diversity of the caulimoviruses reported in dahlia (6,8), we further investigated the Lithuanian dahlias for the presence of various caulimoviruses using species-specific primers and polymerase chain reaction.

The double-stranded DNA genome of DMV was characterized at the molecular level by Richins and Shepherd (10) and the genomic sequences are available in GenBank. In addition to DMV, two other distinct caulimovirus species, tentatively referred to as DMV-D10 (6) and Dahlia common mosaic virus (DCMV) (8) have been reported to infect dahlia. DMV-D10 was found to exist as an endogenous sequence in dahlia (5). Recent surveys of dahlia in the United States, the Netherlands, and New Zealand showed the widespread occurrence of DMV-D10 and DCMV with lower levels of DMV (4,7,9).

Symptomatic dahlia plants suggestive of virus infection were observed during the summer months of 2008 in the Botanical Garden of Vilnius University, Vilnius, Lithuania. Four dahlia cultivars were tested for the caulimoviruses. These were Everest, a Russian cultivar; Zvaigznite, a Latvian cultivar; and two Lithuanian cultivars, Miltinis and Sypsena.

For electron microscopy, a drop of double-distilled water was placed on a grid and a freshly cut symptomatic dahlia leaf was dipped into the drop of water. After 2 min, the excess water was removed using a filter paper and preparations were negatively stained with a drop of 3% uranyl acetate solution. Virus particles were visualized using a JEM-100S electron microscope.

Total DNA extraction of leaves was done as described by Lee et al. (2). Primer pairs specific to each of the three caulimoviruses, DMV (10), DMV-D10 (6), and DCMV (8), were used in polymerase chain reaction. PCR reactions were done separately for each of the species-specific primer pairs. The primer pair specific to the ORF I of DMV-D10 was 5'-CTGTTTTTCTGTGTTTCTACTGG-3' and 5'-ATGGATCGTAAAGATT-3'. The primer pair specific for the coat protein gene of DCMV consisted of 5'- GGATCCTCATTCTGAGTCTTC-3' and 5'- CATATGGCCACCCAAATGACC-3'. To detect DMV, a primer pair derived from the reverse transcriptase gene was used and consisted of 5'-ATGAGTATGCCTCACAGCAA-3' and 5'-TGACCATGCTTCTAACTGT-3'. PCR products were analyzed by agarose gel electrophoresis.
Symptoms included systemic mosaic, mottling and veinal chlorosis (Fig. 1). Electron microscopic examination revealed virions resembling the morphology of caulimovirus virions (Fig. 2). PCR testing showed that all four varieties tested were positive for DMV-D10 and DCMV. DMV-D10 was shown to exist as an endogenous sequence (5). In addition to DMV-D10 and DCMV, Zvaigznite was found positive for DMV. The identity of each of the amplicons was verified by cloning and sequencing. Sequence comparisons showed the sequences obtained in this study were highly identical (92 to 96%) with those available in GenBank.

In addition to their wide spread distribution in the United States and the Netherlands (4,7), dahlia-associated caulimoviruses were recently reported from New Zealand (9). Continued surveys followed by testing to determine their relative incidence is necessary in order to eliminate infected stocks and to generate virus-free breeding and propagative material.
Literature Cited