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Dahlia Mosaic Virus: Molecular Detection and Distribution in Dahlia in the United States

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Abstract. **Dahlia is an important ornamental crop in the U.S. The economic value of the crop is often affected by viral diseases. Of several viruses that infect dahlia, dahlia mosaic virus (DMV) is of most concern. However, little or no information is available about its distribution. A survey of dahlias in several states in the U.S. was carried out during 2003 and 2004. Samples from CA, GA, MD, ME, MT, NM, PA, OR, and WA were tested for DMV. To develop a molecular detection assay, the viral genome was cloned and sequenced and based on the sequence information, DMV-specific primers were used in a PCR-based assay. DMV was detected in >90% of the samples tested. Based on the detection of DMV, a wide range of symptoms were found to be associated with DMV infection. A real-time PCR assay was adapted for rapid detection of DMV. Considering its widespread occurrence, steps are needed to limit its further spread. An effective intervention program would include use of virus-free material to minimize its impact. Availability of a rapid and sensitive detection method such as the once described should facilitate not only production of virus-free dahlias but elimination of virus infected material from breeding and propagating stocks. This is the first report of a survey to determine the extent of DMV incidence in dahlias.**

Dahlia (Genus *Dahlia,* Family *Compositate*) is an important ornamental crop valued for its flowers, and is widely grown in Australia, Canada, Japan, New Zealand, United Kingdom, United States, and several other countries. The economic value of dahlia to the U.S. floriculture industry is significant and probably is worth millions of dollars (www.dahlia.org). Dahlia production is actively pursued by numerous commercial growers, retailers, home gardeners, and dahlia trial gardens (Hines, 2004). The production and propagative activities involving seed, tubers and cuttings across the country and the significant movement of the plant material as part of the commerce, facilitate movement of pests and pathogens, especially viruses. Several viruses are known to infect dahlia. The more commonly found viruses are cucumber mosaic virus (CMV), dahlia mosaic virus (DMV), impatiens necrotic spot virus (INSV), tobacco streak virus (TSV), and tomato spotted wilt virus (TSWV) (Lobenstein et al., 1995).

DMV is an important viral pathogen of dahlias in the U.S. and several parts of the world (Brunt, 1971). It was first reported in *Dahlia pinnata* from Germany in 1928. DMV is reported to be transmitted by several species of aphids in a nonpersistent manner. The host range and some properties of DMV were described by Brunt (1971). A member of the genus *Caulimovirus* of family *Caulimoviridae*, the genome of DMV consists of a circular double stranded-DNA, about 7 kb in size (Richins and Shepherd, 1983). Previous work on dahlia viruses included description of symptomatology, evaluation of various hosts for virus propagation, role of various insects in transmission and production of virus-free dahlias (Albouy et al., 1992; Brierley, 1951; Brierley and Smith, 1950; Mowat, 1980; Mullen and Schlegel, 1978; Wang et al., 1988). The state of the art of DMV research was summarized by Lobenstein et al. (1995). Information on the molecular biology of the virus is limited. With the exception of a report on the physical map of DMV genome (Richins and Shepherd, 1983), and a recent report on the partial sequence information (Nicolaisen, 2003), very little is known about the disease or its incidence in the U.S.

A practical and effective management program to eliminate viruses of ornamentals depends on accurate and rapid identification of the virus and knowledge of its prevalence in various parts of the country (Pappu et al., 2003). However, there is no information on the extent of incidence of DMV in dahlias in the U.S. Moreover, symptomatology of DMV in U.S. dahlias is sparse and information on the DMV symptoms based on confirmation of the association of DMV for a given symptoms type is not available for dahlias grown in the U.S. In a preliminary survey, we found that the DMV is the most prevalent virus of dahlia (Pappu et al., 2004). As a result, we undertook testing of several dahlias collected from several states in the U.S. We cloned and sequenced part of the DMV genome and based on the sequence information, A PCR-based assay was used for specific detection of DMV in dahlias. Using this test, description of various symptoms produced by DMV has been collated. We report the results of the survey for DMV incidence in dahlias using a PCR-based detection assay.

Materials and Methods

Plant materials. Leaf samples from dahlia plants showing symptoms suggestive of virus infection were collected from various parts of the country (Table 1). Samples were collected from commercial gardens, retail outlets, trial gardens, and backyard gardens of individual hobbyists. Leaves were kept in Ziploc bags and stored at 4 °C until tested for the presence of DMV. Sampling was done during the spring and summer of 2003 and 2004. Cultivars that were found positive for DMV are listed in Table 2. Besides DMV, samples were also tested for the presence of CMV, INSV, and TSWV using commercially available ELISA kits (Agdia Inc., Elkart, Ind.).

Cloning of DMV genome. Initial cloning of the DMV genome was accomplished by using primers from conserved region of ORF V of two caulimoviruses, cauliflower mosaic virus and figwort mosaic virus. Standard molecular biology procedures were used for cloning and identification of recombinant clones (Sambrook et al., 1989). Nucleotide sequences were determined at the Sequencing Core Facility of the Washington State University. Sequence searches were done using BLAST (Altschul et al., 1990) to identify related sequences in GenBank (Benson et al., 1996).

Sample preparation for nucleic acid amplification. Total DNA extractions from infected dahlias were made following a modified Dellaporta method (Presting et al., 1995). Each leaf sample (about 100 mg) was ground using mortar and pestle with 1 mL of buffer (100 mM Tris, pH 8.0, 50 mM EDTA, 500 mM NaCl, and 10 mM mercaptoehanol). The extract was transferred to a 1.8 mL microfuge tube to which 140 µL of 10% SDS was added. The sample was mixed by inversion and incubated at 65 °C for 15 min. Following the incubation, 250 µL of 8 M postassium acetate was added, mixed by inversion and kept on ice for 20 min. The samples were then centrifuged at 15,000 rpm for 10 min. The supernatant was transferred to a fresh microfuge tube containing 600 µL of isopropanol. The sample was mixed by inversion and kept on ice for 10 min, followed by centrifugation at 15,000 rpm for 10 min. The supernatant was discarded and the pellet was washed with 500 µL of 70% ethanol. The pellet was dried in SpeedVac and final DNA pellet was resuspended in 100 µL of water and 1:5 dilution was used in PCR. Ten-fold dilutions of total plant DNA were used to estimate the limit of detection.

Polymerase chain reaction (PCR). Primers specific to the ORF 4 of DMV genome were used. The primer pair consisted of 5'-TGC ATA AAA TGA GTT CTA TC-3' and 5'-TGA ACT TGT TCA TCA TTA TC-3'. The PCR reaction included (final concentration), 1X BRL PCR

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Fig. 1. Symptoms of dahlia mosaic virus in dahlia. (**A**) Seedlings may start showing varying degrees of mosaic and/or chlorotic spots. (**B**) Veinal chlorosis. (**C**) Typical mosaic symptoms. (**D**) Systemic chlorosis and stunting of new foliage. (**E**) Stunting and severe deformation of foliage. (**F**) Chlorosis and mottling.

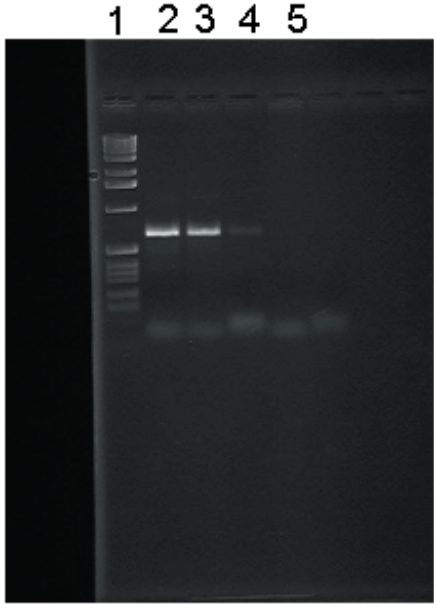

Fig. 2. Agarose gel electrophoresis of dahlia mosaic virus amplicons. PCR products were analyzed by agarose gel electrophoresis. Lane 1 = 1 kb ladder; lanes 2 to 4 = PCR products from total plant DNA diluted 1:10; 1:100 and 1:1000; lane 5 = healthy control.

Buffer, 0.15 mM dNTPs, 2 mM $MgCl_2$, and 0.6 µM of each of the above primers and 2 units of Taq Polymerase (Promega Corp.), in a total reaction volume of 20 µL. The PCR profile consisted of 40 cycles with each one comprising of 94 °C for 30 s, 60 °C for 20 s and 72 °C for 40 s. PCR products were analyzed by 1.0% agarose gel electrophoresis in TAE buffer (Sambrook et al., 1989).

Real-time PCR. Total plant DNA from infected plant was diluted 10- fold up to 1:1000 and the PCR reaction consisted of 3 µL of DNA template, 20 µM of primer V416, 20 µM of primer C1070, 12.5 µL of IQ SYBR Green Supermix (Bio-Rad) and 7.5 µL of sterile distilled water. The PCR profile consisted of one cycle of 94 °C for 5 min., followed by 40 cycles, with each cycle programmed for the following temperature regimes: 94 °C for 30 s, 60 °C for 20 s and 72 °C for 40 s.

Results

Symptomatology of DMV. A wide range of symptoms could be attributed to DMV infection. These included mosaic, chlorotic spots, generalized chlorosis of leaves, systemic chlorosis, deformation of newly emerged foliage, and stunting of infected plants. Some of these symptoms are shown in Fig. 1. There was no correlation between cultivars and a specific type of symptom.

Detection of DMV by PCR. The primer pair, derived from the ORF4 of DMV, when used in PCR, consistently produced a PCR amplicon of expected size of about 500 bp from infected dahlias and not from uninfected plants (Fig. 2). The test was found to be specific for DMV. PCR of 10-fold dilution series of total plant DNA from infected material showed that DMV could be readily detected in 1:100 dilution, whereas, 1:1000 dilution also revealed DMV-specific amplicon that is not readily visible in the picture (Fig. 2, lane 4). The same primer pair was used to develop a real-time PCR assay for DMV detection. DMV could be detected in dilutions of up to 1:1000 of total plant DNA. No amplification could be seen in DNA from uninfected plants (Table 1).

Incidence of DMV. A total of 156 individual plants of several different varieties from several states were tested, and 85% of the samples tested were positive for DMV (Table 2). None of the samples were found infected with CMV, INSV or TSWV. DMV was detected in dahlias from all the states that were surveyed and the virus was present in at least one sample of each lot from each state.

Discussion

Several viruses are known to infect dahlias which tend to spread easily through propagating material. Based on a preliminary testing of dahlias from several states (Pappu et al., 2004), we undertook an extensive survey of dahlias for the presence of DMV. As part of this effort, a portion of the DMV genome was cloned and sequenced. Based on the sequence data, the primer pair, derived from ORF4 was used to develop a DMV-specific PCR assay. The same primer pair was used in a real-time PCR assay to evaluate their specificity in detecting DMV in plant tissue. Based on the testing, it was found that at least one sample from every lot we tested had DMV and samples from all the states we tested were infected with DMV suggesting widespread occurrence of this virus in the U.S. Both PCR and real-time PCR methods are rapid and sensitive. Using a different set of primers, Nicolaisen (2003) reported a PCR-based assay for detection of DMV.

The DNA extraction procedure used is inexpensive and does not need specialty chemicals or columns. The entire procedure including sample preparation to PCR to gel electrophoresis can be completed in a day of 8 h using traditional PCR, and even lesser time if real-time PCR is used. Among the three methods of detection of DMV, namely, ELISA, traditional PCR and real time PCR, ELISA may be the most inexpensive method, followed by traditional PCR, whereas real time PCR requires high initial investment in the equipment. In the absence of a reliable and specific serological assay for DMV detection, the PCR assay should be valuable in screening dahlias for DMV. An ELISA-based assay is being developed for the detection of DMV.

Table 1. A real-time PCR test for the detection of dahlia mosaic virus.

Sample[z]	Dilution	Ct value
Infected plant	1:10	18
Infected plant	1:100	22
Infected plant	1:1000	28
Uninfected plant	1:10	38

[z]Total plant DNA was extracted from infected and uninfected plants and was used in PCR. Primers used were same as in Fig. 2. A 10-fold dilution series of DNA from infected plant was used to determine the sensitivity of the test. Ct refers to comparative threshold cycle which is the cycle number during PCR when the fluorescence from the newly synthesized DNA amplicon exceeds the background amplification.

Table 2. List of dahlia samples that were positive for dahlia mosaic virus.

No.	Variety	Source (state)
1	Jack-O-Lantern	MD
2	CG Coral	MD
3	Laredo	MD
4	CG Raven	MD
5	Kenora Sunset	MD
6	Spirit of Elsie Huston	MD
7	Scarborough Brilliant	MD
8	Lovely Lama	MD
9	Twinny	MD
10	CG Coral Top	MD
11	313 CG Regal	MD
12	Allie White	MD
13	Wildwood Marie	WA
14	Jessie G	WA
15	Procho Ami	WA
16	CG Paragon	CA
17	CG Regal	CA
18	Magic Moment	MD
19	Windhaven Highlight	MD
20	Sea Fuego	MD
21	Alpen Steve	MT
22	Alpen X	MT
23	Alpen Joy	MT
24	Evening Lady	MT
25	Show N Tell	GA
26	Creekside Ruth	GA
27	Kenora Sunset	PA
28	CG Regal	PA
29	Magic Moment	PA
30	Unknown variety	PA
31	Kenora Sunset	NM
32	Amy Star	ME
33	Honka dahlia	ME

The potential impact of DMV infection on the economic value of dahlias is not known. DMV infection is known to adversely affect the plant vigor thus reducing the quality and number of flowers a plant produces. Plants infected at early age or conditions that pre-dispose them for expression of symptoms usually results in stunting of plants or stunting and deformation of new foliage leading to overall poor growth of the plant. The high incidence of DMV highlights the need for increased awareness of the virus and the need to take steps by floriculture industry to reduce its further spread. Detection followed by removal of infected material and avoiding propagation infected material, combined with restricted movement of infected material can be very effective in reducing the further spread of DMV in dahlias. Other management practices such as tissue culture (Mullen and Schlegel, 1978; Wang et al., 1988) and heat treatment of tubers may be effective in eradicating the infection.

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