

Full Length Research Paper

Caulimoviral sequences in *Dahlia variabilis* in Egypt

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The presence of *Dahlia mosaic virus* D10 (DMV-D10) was confirmed for the first time in dahlia (*Dahlia variabilis*) in Egypt. DMV-D10 was recently described as a caulimovirus that exists as an endogenous pararetroviral sequence (EPRS). DMV-D10 was confirmed by amplification of the ORF1 (encoding for the movement protein) using species specific primers (D10F1/R1). The expected size (900 bp) was amplified from 36 samples with no evidence of infection with either DMV or DCMV. The same dahlia plants were tested for the presence of CMV, INSV, TSV, and TSWV and they were all negative. Sequence comparisons of the Egyptian DMV-D10 ORF1, GenBank accession HM007162, amplified from these samples revealed that the amplicon had the highest sequence identity (96%) with that of DMV-D10 (isolated from US dahlia cultivars). Cluster dendrogram based on the amino acid sequences of movement protein of all known caulimoviruses placed D10-US (isolated from US dahlia cultivars), D10 – NZ (isolated from New Zealand dahlia cultivars), D10-DC (isolated from *D. coccinea*) and D10-Egypt (isolated from Egyptian dahlia cultivars) in one clade.

Key words: *Dahlia variabilis*, endogenous pararetroviral sequence, DMV-D10.

INTRODUCTION

Dahlia (*Dahlia variabilis*) is an important bulb crop worldwide used as garden or potted ornamentals. Dahlias are mainly produced using vegetative propagation. One of the most important diseases affecting dahlia is Dahlia mosaic. Symptoms associated with Dahlia mosaic vary considerably according to cultivar, but most often consist of mosaic, veinbanding, chlorosis, leaf malformation, flower-color breaking and stunting (Nicolaisen, 2003; Pahalawatta et al., 2008a). Some of the *Dahlia* cultivars,

on the other hand, may show no symptoms of viral infection (Nicolaisen, 2003).

Molecular detection tools have revealed three major caulimoviruses associated with *Dahlia mosaic* disease including Dahlia mosaic virus (DMV), (Brunt, 1971, Richins and Shepherd, 1983) and Dahlia common mosaic virus (DCMV), (Pappu et al., 2008a) and a third caulimovirus designated as DMV-D10 was found to be distinct from DMV (Pahalawatta et al., 2008a; Eid et al., 2009). The genome structure and organization of DMV-D10 is ca. 7.0 kb in size and shares many of the features of the members of the genus *Caulimovirus*, such as the presence of genes potentially coding for the movement protein, the inclusion body protein, the reverse transcriptase (RT), and an intergenic region consisting of a potential 35S promoter homologue. However, the D10 genome differed from *Dahlia mosaic* caulimovirus and other known caulimoviruses in that the aphid transmission factor (ATF) was absent and the putative coat protein contained a C-terminal deletion and was fused in-frame with the RT region (Pahalawatta et al., 2008b).

DMV-D10 exists in all plant parts (leaves, roots, seeds, flower petals and pollen). It could be detected in tissue

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Abbreviations: DMV, Dahlia mosaic virus; DCMV, Dahlia common mosaic virus; DMV-D10, *Dahlia mosaic* virus D10; EPRS, endogenous pararetroviral sequences; ATF, aphid transmission factor; CMV, cucumber mosaic virus; INSV, impatiens necrotic spot virus; TSV, tobacco streak virus; TSWV, tomato spotted wilt virus; TVCV, tobacco vein clearing virus; BSV, banana streak virus; PVCV, petunia vein clearing virus; RT-PCR, reverse transcription polymerase chain reaction; ORF, open reading frame.

Table 1. Specific primers used for each of the three caulimoviruses infecting dahlia.

Primers	Sequence (5'-3')	Annealing temperature (°C)	Expected size
D10 F1 D10 R1	ATGGATCGTAAAGAT T CTG TTT TTC TGT GTT TCT ACT GG	50	900 bp
DMV F1 DMV R1	ATG AAT ATC TTA GAA AGG AA CTT AAT CCT TAA GTT ATC AA	50	939 bp
DCMV F2 DCMV R2	ACA GGA GTT AAA AGC TGG TCG AGG ATA TTG TTG TTG	48	646 bp
DCMV CP F DCMV CP R	GGATCCTCATTCTGAGTCTTCGTCTTC CATATGGCCACCCAAATGACC	59	1517 bp

culture-derived dahlia plants. More-over, DMV-D10 is transmitted through seed to 100% of the progeny plants resulting in establishment of infection and symptom expression (Pahalawatta et al., 2007a,b). Presence of D10 sequences was reported in over 98% of the samples of numerous dahlia varieties tested from several parts of the world (Pappu et al., 2005; Pahalawatta et al., 2007b).

Integration of viral DNA into bacterial and animal host chromosomes is a common occurrence. There are limited reports of integration of viral sequences in a plant genome. The integrated viral sequences or EPRVs described so far were all derived from viruses with DNA genomes or from viruses that have a DNA phase in their replication cycle. These include the single stranded DNA geminiviruses (Bejarano et al., 1996; Ashby et al., 1997) and the double-stranded DNA viruses (reviewed by Harper et al., 2002; Staginnus and Richert-Poggeler, 2006). The Caulimoviridae family consists of six genera, with plant viruses that replicate by reverse transcription (Hull, 2001). Four viruses, representing three of the genera in this family, were reported as integrants in their respective host plant genomes. These include *Banana streak virus* (BSV) in *Musa* spp. (Harper et al., 1999; Ndowora et al., 1999), *Petunia vein clearing virus* (PVCV) in *Petunia* spp (Richert-Poggeler et al., 1997, 2003; Harper et al., 2003), *Tobacco vein clearing virus* (TVCV) in *Nicotiana* spp. (Jakowitsch et al., 1999; Lockhart et al., 2000; Gregor et al., 2004), and *Rice tungro bacilliform virus* (RTBV) in *Oryza* spp (Kunii et al., 2004). Although integration events were observed for these viral sequences, many of these viruses do not encode an integrase function. DMV-D10, on the other hand, is capable of initiating infection and produce disease symptoms (Pahalawatta et al., 2008b).

The integration of viral sequences in host genomes represents the application of maximum economy in nature where viruses can be spread out worldwide without being noticed and neutralizes the need for virus-vector transmission. Additionally, tools such as meristem tip culture used for virus elimination are ineffective in virally-integrated genomes such as BSV (Geering et al., 2005) and DMV-D10 (Pahalawatta et al., 2008b).

DM symptoms have been observed on *Dahlia* plants grown at the Faculty of Agriculture, Cairo University since March 2008. Infected plants showed mild mosaic, stunting, leaf deformation and color breaking of the flowers. PCR was performed using the primers pairs (DMV F1/R1, DCMV CPF/R, DCMV F2/R2, and D10F1/R1) to determine the incidence of the three caulimoviruses DMV, DCMV and DMV-D10, respectively. This study reports the sequence of ORF1 of the Egyptian isolate of DMV-D10.

MATERIALS AND METHODS

Plants displaying mosaic symptoms (46 out of 153 healthy-looking plants) indicative of viral infection were observed on the campus of the Faculty of Agriculture, Cairo University during the month of March 2008. Total RNA was extracted (Roche Molecular Biochemicals, cat. No 2033674) and tested for the following viruses by RT-PCR: Cucumber mosaic virus (CMV), Impatiens necrotic spot virus (INSV), Tobacco streak virus (TSV), and Tomato spotted wilt virus (TSWV). None of them were found to be present in the samples. Total DNA was extracted (Echevarría-Machado et al., 2005) and tested for three caulimoviruses associated with dahlia: *Dahlia mosaic virus* (DMV), *Dahlia common mosaic virus* (DCMV), and an endogenous plant pararetroviral sequence DMV-D10.

PCR was performed using the primers pairs (DMV F1/R1, DCMV CPF/R, DCMV F2/R2, and D10F1/R1) (Table 1) to detect the incidence of the three caulimoviruses DMV, DCMV and DMV-D10, respectively. Each 20 µl PCR reaction contained 1 µl of the total nucleic acid extract, 1X PCR buffer (20 mM Tris, pH 8.4 and 500 mM KCl), 150 µM dNTP mix, 2 mM MgCl₂, and 0.6 pmole each of forward and reverse primers, 12.8 µl sterile H₂O, and 0.1 µl Taq DNA polymerase. The amplification was performed in a DNA thermal cycler (BioRad, Hercules, CA) with initial denaturation at 94°C for 4 min followed by 50 cycles of 94°C for 30 s, the required annealing temperature (Table 1) based on primer pair used for 20 s, and 72°C extension step determined based on the size of the amplicon to be synthesized at the rate of 1000 bp /min, and terminated by a final incubation at 72°C for 7 min.

PCR products (7.5 µl) were analyzed by agarose gel electrophoresis (1.2%) in 0.5 X TAE (Tris-Acetate-EDTA) buffer. Amplicons. PCR amplicon was cloned into pGEM-T (Promega, Madison, WI) by following the manufacturer's instructions. The plasmids DNA were sequenced using the ABI Prism Sequencing System was at the Molecular Biology Core Laboratory of the Washington State University, Pullman, WA. Sequences obtained were compared to



Figure 1. A) Mosaic symptoms on leaves; B) diseased flowers, showing color breaking, (B-1, 2 and 3) of infected *Dahlia* plants growing at the Faculty of Agriculture, Cairo University; and C) healthy control.

those available in GenBank and phylogenetic analysis was done using MEGA4 (Tamura et al., 2007).

RESULTS AND DISCUSSION

The presence of DMV-D10 was confirmed by amplification of the ORF1 (encoding for the movement protein) using species specific primers (D10F1/R1). The expected size (900 bp) was amplified from 36 samples and there was no evidence of infection with either DMV or DCMV (Figure 2). The same dahlia plants were tested for the presence of CMV, INSV, TSV, and TSWV and they were

all negative. Therefore the symptoms of mild mosaic, stunting, leaf deformation and colour breaking of the flowers observed on the 36 samples tested could be due to DMV-D10 infection (Figure 1). There are several reports showing that EPRVs are not neutral components of plant genomes but can potentially contribute to pathogenicity (Geering et al., 2005; Lockhart et al., 2000; Richert-Poggeler et al., 2003). It was shown that some EPRVs (for example, PVCV) could give rise to episomal molecules and cause symptoms under certain conditions (Richert – Poggeler et al., 2003).

Sequence comparisons of DMV-D10 ORF1 amplified from these samples (GenBank accession HM007162)

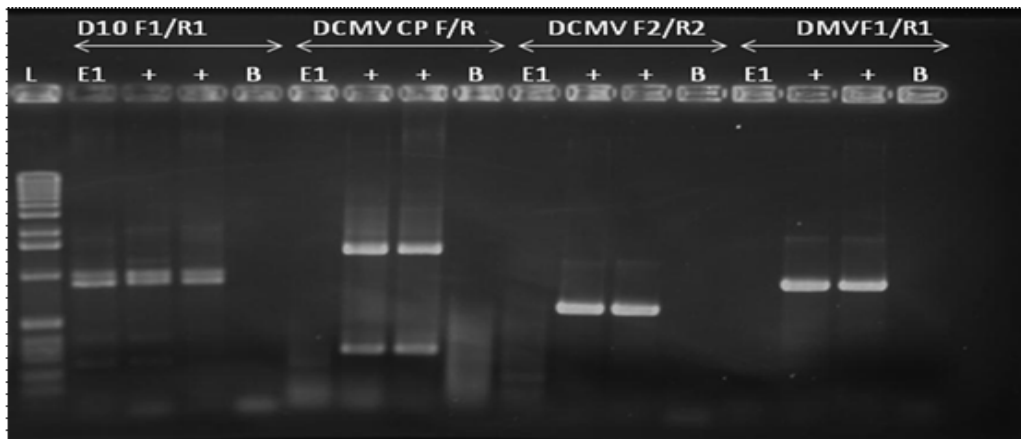


Figure 2. Agarose gel electrophoresis of polymerase chain reaction (PCR) products amplified with D10 F1/R1 (ORF1), DCMV CP F/R, DCMV F2/R2, DMV F1/R1. **B**, Negative control; **E**, Egypt sample; **+**, positive control; **L**, 1-kb DNA ladder.

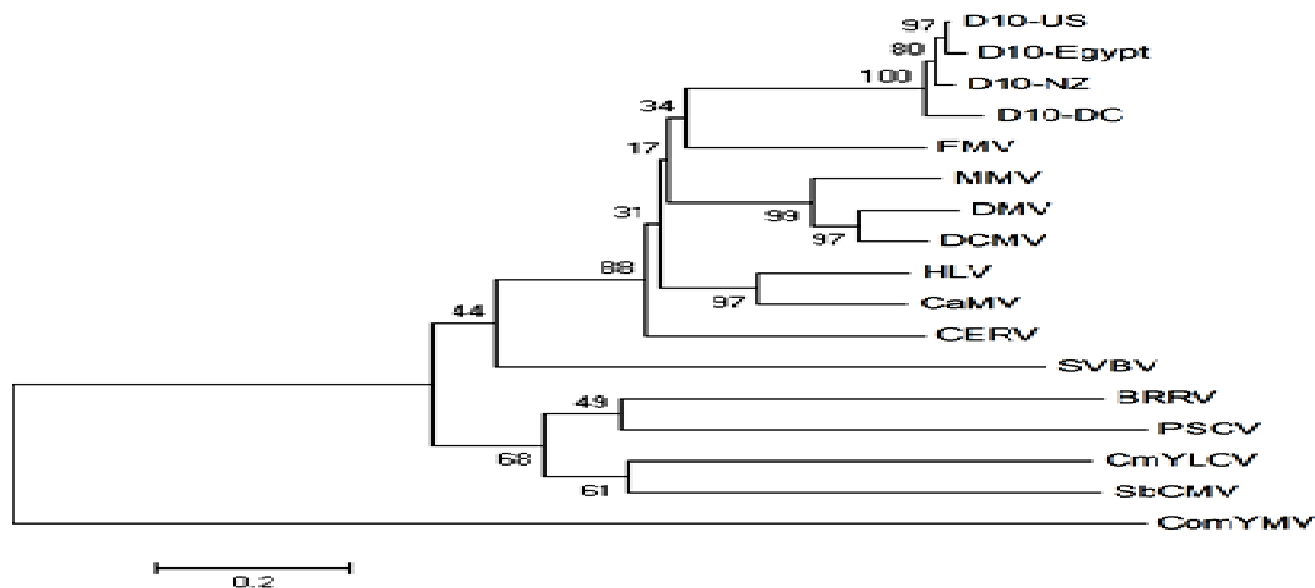


Figure 3. Phylograms drawn from Clustal W alignments of movement protein from selected members of the family *Caulimoviridae* compared with those of DMV, DMV-D10 (D10-US, D10-NZ, D10-DC, D10-Egypt) and DCMV. BRRV, blueberry red ringspot virus; CaMV, cauliflower mosaic virus; CERV, carnation etched ring virus; CmYLCV, cestrum yellow leaf curling virus; ComYMV, commelina yellow mottle virus; FMV, figwort mosaic virus; HLV, horseradish latent virus; MMV, mirabilis mosaic virus; PSCV, peanut chlorotic streak virus; SbCMV, soybean chlorotic streak virus; and SVBV, strawberry vein banding virus. Bootstrap values are indicated at branching points in the phylogram as a percentage of 1,000 iterations.

revealed that the amplicon had the highest sequence identity (96%) with that of DMV-D10 (isolated from US dahlia cultivars) confirming the presence of DMV-D10 in dahlia samples. Cluster dendrogram based on the amino acid sequences of movement protein of all known caulimoviruses put D10-US (isolated from US dahlia cultivars), D10 -NZ (isolated from New Zealand dahlia cultivars), D10-DC (isolated from *D. coccinea*) and D10-Egypt (isolated from Egypt dahlia cultivars) in one clade (Figure 3).

The presence of DMV-D10 in *Dahlia* in Egypt suggests the need to test for caulimovirus for a better understanding of the incidence and distribution of these viruses in *Dahlia* in order to develop virus management programs to reduce their spread.

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