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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 dendogram 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; **EPRV,** 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	ATGGATCGTAAAGAT T	50	900 bp
D10 R1	CTG TTT TTC TGT GTT TCT ACT GG		
DMV F1	ATG AAT ATC TTA GAA AGG AA	50	939 bp
DMV R1	CTT AAT CCT TAA GTT ATC AA		
DCMV F2	ACA GGA GTT AAA AGC TGG	48	646 bp
DCMV R2	TCG AGG ATA TTG TTG TTG		
DCMV CP F	GGATCCTCATTCTGAGTCTTCGTCTTC	59	1517 bp
DCMV CP R	CATATGGCCACCCAAATGACC		

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-Pöggeler 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 $MgCl_2$, and 0.6 pmole each of forward and reverse primers, 12.8 µl sterile H_2O, 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 – Pöggeler et al., 2003).

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

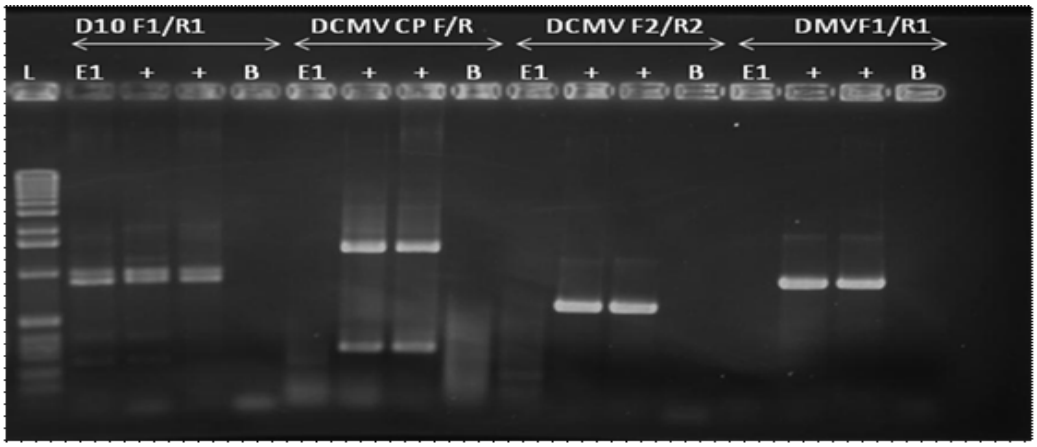

Figure 2. Agarose gel electophoresis of polymerase chain reaction (PCR) products amplified with D10 F1/R1 (ORFI), DCMV CP F/R, DCMV F2/R2, DMV F1/R. **B,** Negative control; **E,** Egypt sample; **+,** positive control; **L,** 1-kb DNA ladder.

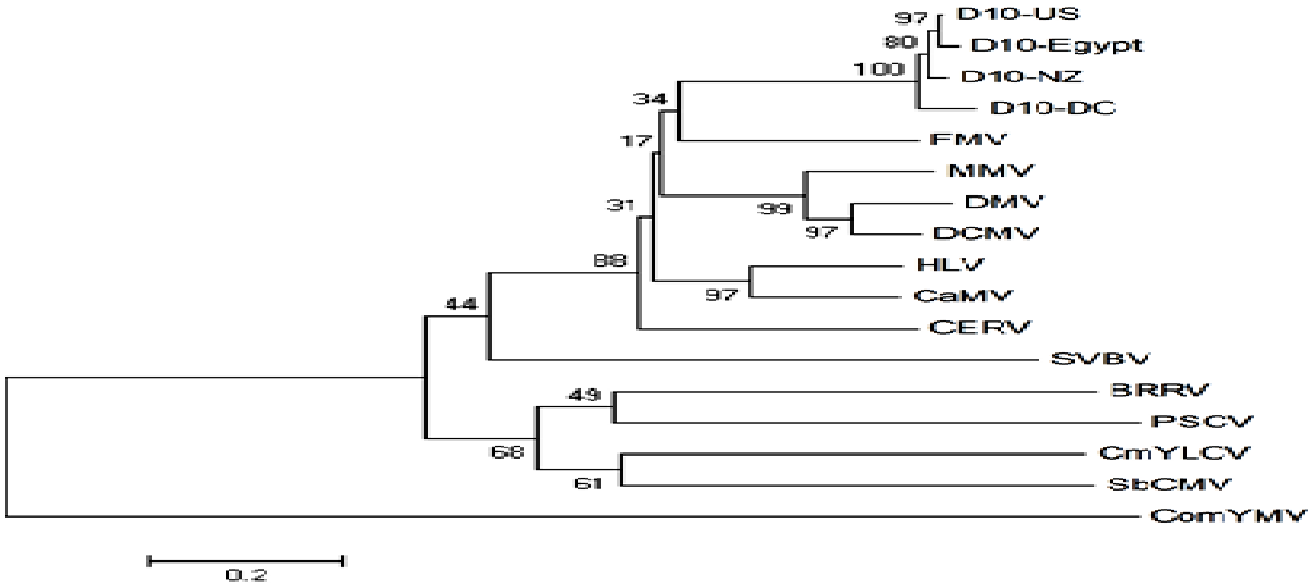

Figure 3. Phylograms drawn from Clustal W alignments of movement protein from selected members of the family *Caulimoviridae* compared with those of DMV, DMV-D10 (D10-US, D10-NZ, D10-DC, D10-Egypt) and DCMV. BRRV, blueberry red ringspot virus; CaMV, cauliflower mosaic virus; CERV, carnation etched ring virus; CmYLCV, cestrum yellow leaf curling virus; ComYMV, commelina yellow mottle virus; FMV, figwort mosaic virus; HLV, horseradish latent virus; MMV, mirabilis mosaic virus;, PCSV, 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 dendogram 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.

REFERENCES

Ashby MK, Warry A, Bejarano ER, Khashoggi A, Burrell M, Lichtenstein C (1997). Analysis of multiple copies of geminiviral DNA in the geno-

me of four closely related Nicotiana species suggests a unique integration event. Plant Mol. Biol. 35: 313-321.
Bejarano ER, Khashoggi A, Witty M, Lichtenstein C (1996). Integration of multiple repeats of geminiviral DNA into the nuclear genome of tobacco during evolution. Proc. Natl. Acad. Sci. USA, 93: 759-764.
Brunt AA (1971). *Dahlia mosaic* virus. Descriptions of Plant Viruses. p.51.
Echevarría-Machado I, Sánchez-Cach LA, Hernández-Zepeda C Rivera-Madrid R, Moreno-Valenzuela OA (2005). A simple and efficient method for isolation of DNA in high mucilaginous plant tissues. Mol. Biotechnol. 31(2): 129-135.
Eid S, Druffel KL, Saar D, Pappu HR (2009). Incidence of multiple and distinct species of caulimoviruses in dahlia (Dahlia variabilis). Hort. Sci. 44: 1498-1500.
Geering ADW, Olszewski NE, Harper G, Lockhart BE, Hull R, and Thomas JE (2005). Banana contains a diverse array of endogenous badnaviruses. J. Gen. Virol. 86: 11-520.
Gregor W, Mette MF, Staginnus C, Matzke MA, Matzke AJM (2004). A distinct endogenous pararetrovirus family in Nicotiana tomentosiformis, a diploid progenitor of polyploid tobacco. Plant Physiol. 134: 1191-1199.
Harper G, Hull R, Lockhart B, Olszewski N (2002). Viral sequences integrated into plant genomes. Annu. Rev. Phytopathol. 40: 119-136.
Harper G, Osuji JO, Heslop-Harrison JS, Hull R (1999). Integration of banana streak badnavirus into the Musa genome: molecular and cytogenetic evidence. Virology, 255: 207-213.
Harper G, Richert-Pöggeler KR, Hohn T, Hull R (2003). Detection of petunia vein clearing virus: model for the detection of DNA viruses in plants with homologous endogenous pararetrovirus sequences. J. Virol. Methods, 107: 177-184.
Hull R (2001). Matthews Plant Virology, 4th Edition. Academic Press. p. 1056.
Jakowitsch J, Mette MF, Van Der Winden J, Matzke MA, Matzke AJM (1999). Integrated pararetroviral sequences define a unique class of dispersed repetitive DNA in plants. Proc. Natl. Acad. Sci. USA, 96: 13241-13246.
Kunii M, Kanda M, Nagano H, Uyeda I, Kishima Y, Sano Y (2004). Reconstruction of putative DNA virus from endogenous rice tungro bacilliform virus-like sequences in the rice genome: implications for integration and evolution. BMC Genomics, 5: p. 80.
Lockhart BE, Menke J, Dahal G, Olszewski NE (2000). Characterization and genomic analysis of tobacco vein clearing virus, a plant pararetrovirus that is transmitted vertically and related to sequences integrated in the host genome. J. Gen. Virol. 81: 1579-1585
Ndowora T, Dahal G, LaFleur D, Harper G, Hull R, Olszewski NE, Lockhart B (1999). Evidence that badnavirus infection in Musa can originate from integrated pararetroviral sequences. Virology, 255: 214-220.
Nicolaisen M (2003). Partial Molecular Characterization of *Dahlia mosaic* virus. Plant Dis. 87(80): 945-948.
Pahalawatta V, Druffel K, Pappu HR (2007a). Seed transmission of *Dahlia mosaic* virus in Dahlia pinnata. Plant Dis. 91: 88-91.
Pahalawatta V, Druffel KL, Pappu HR (2008b). A new and distinct species in the genus *Caulimovirus* exists as an endogenous plant pararetroviral sequence in its host, *Dahlia variabilis*. Virology, 376: 253-257.
Pahalawatta V, Druffel KL, Wyatt SD, Eastwell KC, PappuHR (2008a). Genome structure and organization of a novel and distinct species of genus *Caulimovirus* (Family *Caulimoviridae*) associated with *Dahlia mosaic*. Arch. Virol. 153: 733-738.
Pahalawatta V, Miglino R, Druffel KL, Jodlowska A, Van Schadewijk AR, Pappu HR (2007b). Incidence and relative distribution of distinct caulimoviruses associated with *Dahlia mosaic* in Dahlia variabilis. Plant Dis. 91: 1194-1197.
Pappu HR, Druffel KL, Miglino R, Van Schadewijk AR (2008a). Nucleotide sequence and genome organization of a new and distinct caulimovirus associated with dahlia mosaic. Arch. Virol. 153: 2145-2148.
Pappu HR, Wyatt SD, Druffel KL (2005). *Dahlia mosaic* virus: Molecular detection and distribution in dahlia in the US. Hort. Sci. 40: 697-699.
Richert-Pöggeler KR, Noreen F, Schwarzacher T, Harper G, Hohn T (2003). Induction of infectious petunia vein clearing (pararetro) virus from endogenous provirus in petunia. EMBO J. 22: 4836-4845.
Richert-Pöggeler KR, Shepherd RJ (1997). Petunia vein clearing virus: a plant pararetrovirus with core sequences for an integrase function. Virology, 236: 137-146.
Richins RD, Shepherd RJ (1983). Physical maps of the genome of *Dahlia mosaic* virus and Mirabilis mosaic virus-two members of the Caulimovirus group. Virology, 124: 208-214.
Staginnus C, Richert-Poggeler KR (2006). Endogenous pararetroviruses: two faceted travelers in the plant genome. Trends Plant Sci. 11: 485-491.
Tamura T, Dudley J, Nei M, Kumar S (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. and Evol. 24(8):1596-1599; doi:10.1093/molbev/msm092.