

Phylogenetic distribution of an endogenous strain of Dahlia mosaic virus in members of Asteraceae

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INTRODUCTION



Dahlia Mosaic Virus (DMV) is a double-stranded DNA viral pathogen belonging to the family Caulimoviridae (Pahalawatta et al., 2008). Symptoms associated with DMV include vein clearing in the leaves (Figure 1a), flower breaking (Figure 1b), and stunted growth (Abdel-Salam et al., 2010). DMV is most commonly observed in horticultural and wild varieties of the genus *Dahlia*. Additionally, a new strain of this virus called DMV-D10 was first observed in *Dahlia variabilis* growing in Egypt (Abdel-Salam et al., 2010).

DMV-D10 is an endogenous pararetrovirus (Pahalawatta, et al., 2008). A pararetrovirus has the ability to integrate its viral sequences into the host plant genome, which can be inherited from parent to offspring (Geering, 2014). DMV-D10 lacks the aphid transmission factor used by that of its closest relatives DMV and DCMV (Dahlia common mosaic virus), and presumably limiting its spread. In addition, DMV-D10 does not have any visible symptoms, which makes it difficult to determine presence or absence of the virus in host plants. Currently, no studies have examined the full extent of DMV-D10 host range. Because DMV-D10 and closely related viruses have only been observed in *Dahlia*, our study aims to determine if there is a relationship among other species related with *Dahlia*.



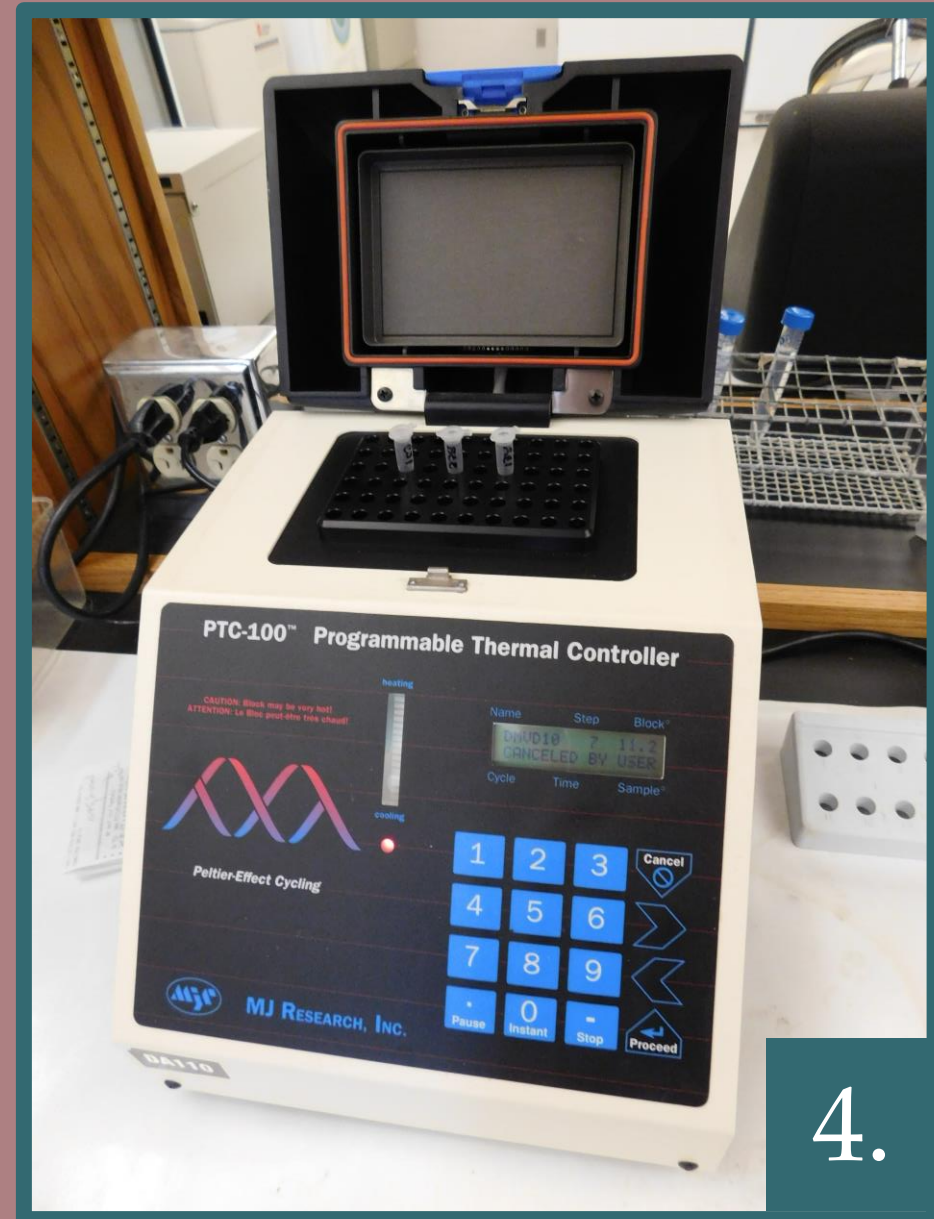
MATERIALS AND METHODS



Seeds for six varieties of five species in five genera within Asteraceae were grown under greenhouse conditions for five weeks (Figure 2). Fresh tissue from one whole, healthy plant (i.e., root, leaves, and stem) was harvested per variety. Tissue was ground in liquid nitrogen into a fine powder for DNA extraction.



DNA was extracted with a Qiagen DNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands) following the Quick Start Protocol (Figure 3) included in the kit. Agarose Gel Electrophoresis was conducted to ensure successful DNA extraction before PCR. Samples were frozen at -20 °C until further analysis.



PCR was conducted with components from a Phusion High-Fidelity PCR Kit (Thermo Fisher Scientific, Waltham, Massachusetts). Samples were placed in a PTC-100 Programmable Thermal Controller (Figure 4). Programming details and movement protein primers were followed according to Abdel-Salam et al. (2010). DNA amplification was performed with a denaturation step at 94 °C and annealing step at 50 °C for 50 cycles, followed by an extension step at 72 °C. Agarose Gel Electrophoresis was conducted with PCR products to analyze presence or absence of a DMV-D10 movement protein gene at 900 bp.

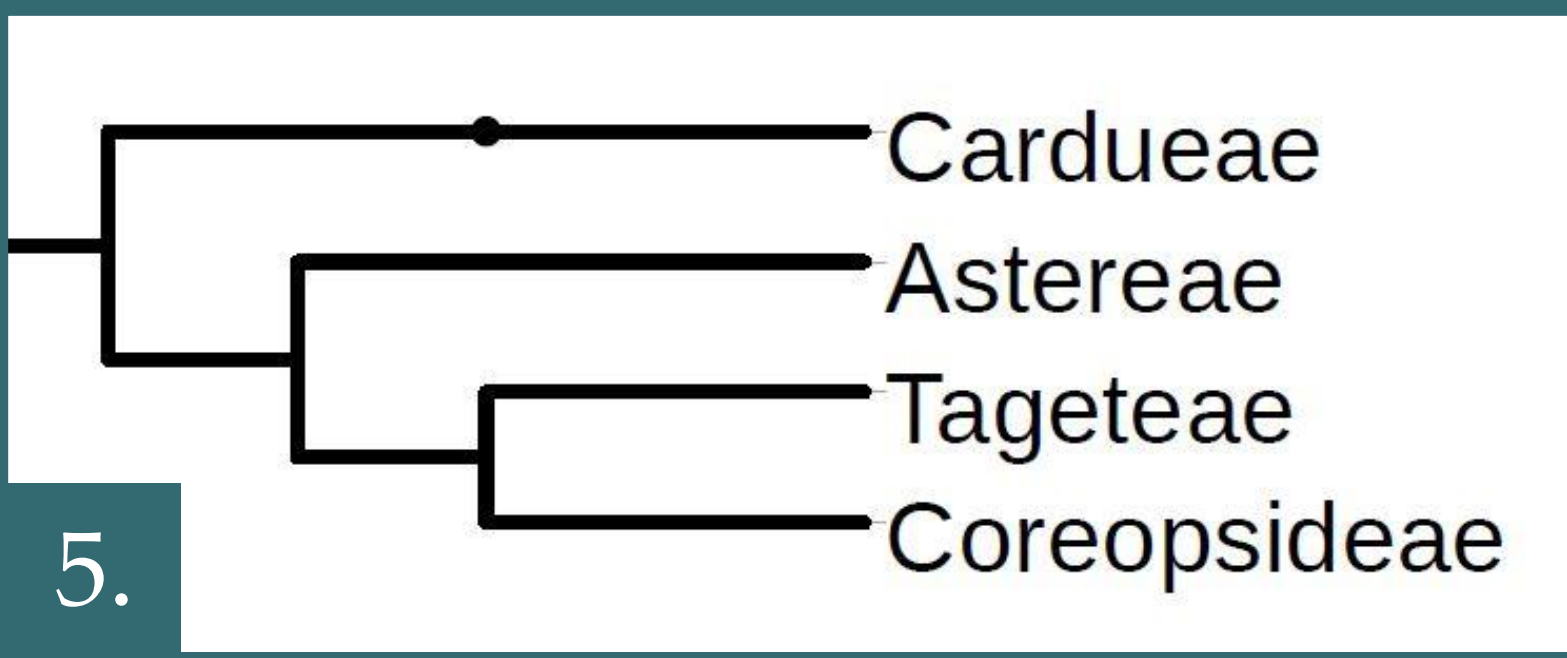
OBJECTIVES & HYPOTHESES

Determine the host range of DMV-D10 by sampling Asteraceae members including *Dahlia variabilis*

- Cosmos bipinnatus* and *Dahlia variabilis* belong to the Coreopsideae tribe in Asteraceae; therefore, we hypothesized DMV-D10 was most likely to be present in these species compared to others based on phylogeny.
- In contrast, we hypothesized members in the Astereae, Cardueae, and Tageteae tribe would be less likely to be infected with DMV-D10 based on phylogenetic relationship with Coreopsideae.

RESULTS

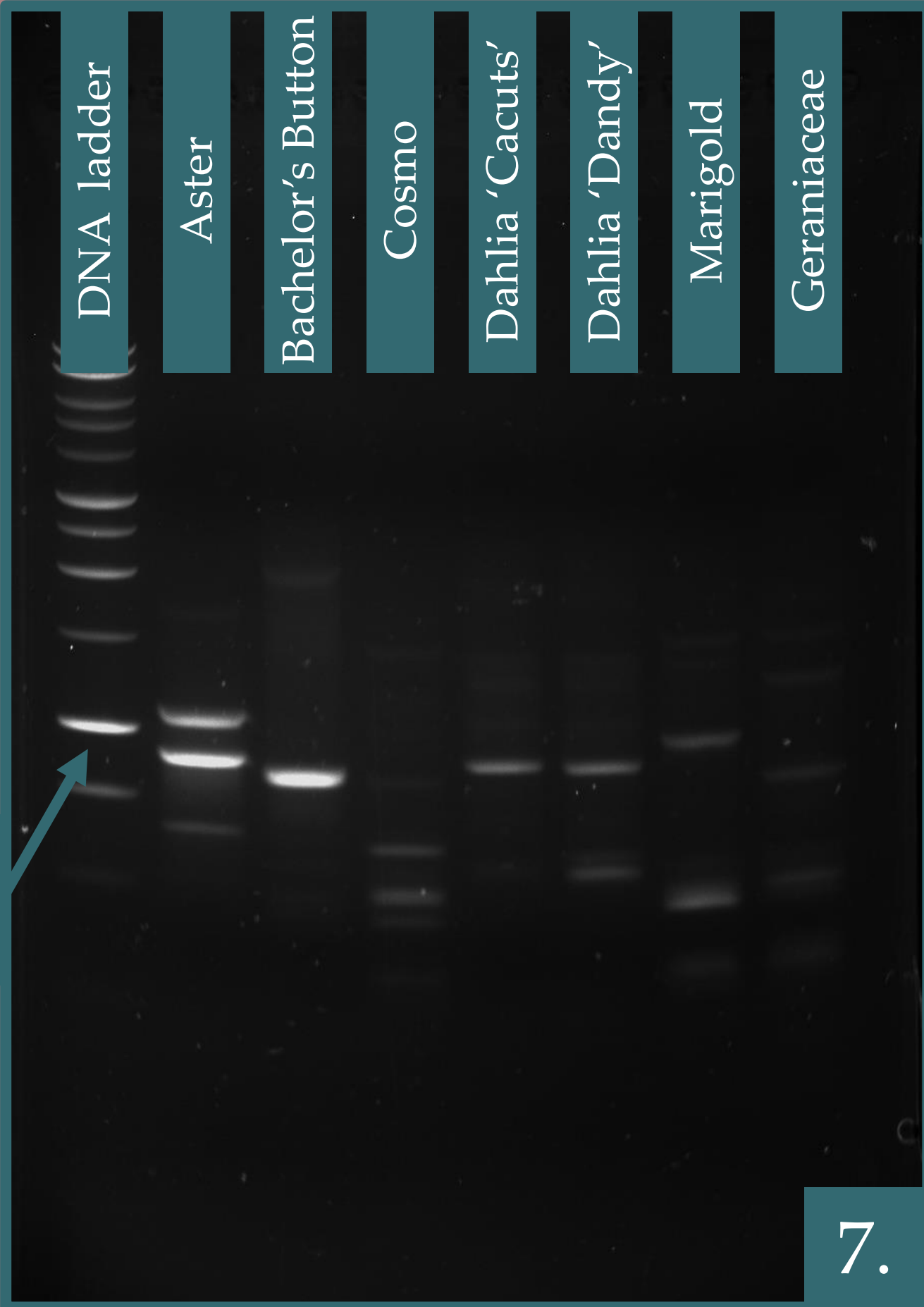
Phylogeny of Asteraceae tribes (Figure 5) (Letunic and Bork, 2011). Table of species in this study indicating tribe, common name, horticultural variety, and presence of a DMV-D10 movement protein (Figure 6).



Tribe	Genus	Species	Common name	Variety	DMV-D10 movement protein
Astereae	<i>Callistephus</i>	<i>chinensis</i>	Aster	'Crego'	Positive
Cardueae	<i>Centaurea</i>	<i>cyanus</i>	Bachelor's Button	'Cyanus Double'	Positive
Coreopsideae	<i>Cosmos</i>	<i>bipinnatus</i>	Cosmos	'Single Sensation'	Negative
Coreopsideae	<i>Dahlia</i>	<i>variabilis</i>	Dahlia	'Cactus'	Positive
				'Dandy'	Positive
Tageteae	<i>Tagetes</i>	<i>erecta</i>	Marigold	'Crackerjack'	Negative



PCR products were analyzed for presence of a DMV-D10 movement protein gene at 900 bp (Abdel-Salam et al., 2010). Results indicate DMV-D10 is more widespread across the Asteraceae family, and not limited to *Dahlia*. Gel electrophoresis results (Figure 7) show bands at 900 bp in Aster (Astereae tribe), Bachelor's Button (Cardueae tribe), and Dahlia 'Cactus' and 'Dandy' varieties (Coreopsideae tribe). However, Cosmo (Coreopsideae tribe), Marigold (Tageteae tribe), and Geraniaceae (the negative control) did not exhibit bands at 900 bp.



DISCUSSION

There was no phylogenetic relationship when determining absence or presence of DMV-D10 in samples. The DMV-D10 movement protein was present in *Callistephus chinensis*, which belongs to the Astereae tribe, *Centaurea cyanus*, belonging to the Cardueae tribe, and *Dahlia variabilis*, belonging to the Coreopsideae tribe. This indicates DMV-D10 or a related DMV virus was present in some samples, but not all of them. Past studies indicate DMV-D10



spreads via vertical transmission from parent to offspring (Pahalawatta et al., 2008). Therefore, this inconsistency of viral infection with relation to phylogeny leads to questions about how this virus is transmitted other than from parent to progeny. It is possible the integration of this virus into the plant genome can be aged based on distribution in related species (Geering and Scharaschkin, 2010).



For instance, one study suggested the integration events of *Badnavirus* (Caulimoviridae) into the plant genome occurred more than 4.6 million years ago when two plant species of *Musa* derived from a common ancestor (Lescot et al., 2008). Therefore, hints of a wider distribution of DMV-D10 indicated in this study may be evidence of long term evolution of this virus within Asteraceae. Contrary to this hypothesis, there could be vectors of transmission that are still unknown. DMV-D10 lacks an aphid transmission factor (Pahalawatta et al., 2008). Therefore, it is possible another insect mode of DMV-D10 transmission has yet to be discovered. Further research is needed to determine how DMV-D10 is spread to other species in the Asteraceae family. Our future research will explore how broad the host range for DMV-D10 is in Asteraceae by having a larger sample size, including more genera of Asteraceae. We will also examine if related viruses are present in samples to provide more background. Additionally, future research will examine if there are physiological symptoms that correspond with DMV-D10 infection. Overall, this could provide us with a better understanding of the biology of this virus in relation to their host plants.



ACKNOWLEDGMENTS

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