Rapid real-time detection of *Dahlia mosaic virus*, *Dahlia common mosaic virus*, and an endogenous control in Dahlias using isothermal AmplifyRP®

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Introduction

Dahlia, a genus of the *Asteraceae* family, contains 42 species with over 20,000 cultivars. They are one of the most beautiful and popular ornamental plants grown worldwide. However, numerous pathogens can cause diseases in dahlias and affect their growth and production. Among them are *Dahlia mosaic virus* (DMV) and *Dahlia common mosaic virus* (DCMV), two distinct DNA viruses in the *Caulimovirus* genus of the *Caulimoviridae* family (1, 2). These viruses may cause yellowing and stunting of plants. Early and rapid detection of the two viruses is important for management of viral disease in dahlias, however, there are limited detection methods presently available. Utilizing the leading isothermal amplification technology, recombinase polymerase amplification (RPA) (3), Agdia Inc. has developed its AmplifyRP® platform for rapid detection of all kinds of plant pathogens (4, 5). In this report, we describe the development of a duplex XRT assay of AmplifyRP® for rapid and real-time detection of both DMV and DCMV along with an endogenous control gene.

Materials and methods

AmplifyRP® primers and probes were designed from conserved sequence regions of both DMV and DCMV and screened for their performance. A pair of primers and a probe were chosen for the final assay due to their specific and sensitive amplification. The AmplifyRP® XRT for DMV & DCMV is optimized to run at 42°C for 20 minutes and the amplification/detection program is barcoded for the portable fluorescence reader AmpliFire® as shown in **Figure 1**. The results are displayed in real-time and the final data can be exported and further analyzed.

As outlined in **Fig. 1**, crude extracts from plant leaf tissues were prepared by grinding them 1:20 (tissue weight/buffer volume) in a GEB-filled buffer bag and then 25 µl of the extract was mixed with 1,000 µl of the Pellet Diluent. Twenty-five microliters of this mix per reaction was added to the reaction pellet and applied for testing with AmplifyRP® XRT for DMV & DCMV. The amplification process and results are displayed on the AmpliFire® screen for the viral gene target through FAM channel and an endogenous plant gene as an internal control through ROX channel. In addition to virus-infected plant samples, synthetic DNA fragments were also tested in the presence of healthy dahlia extracts.

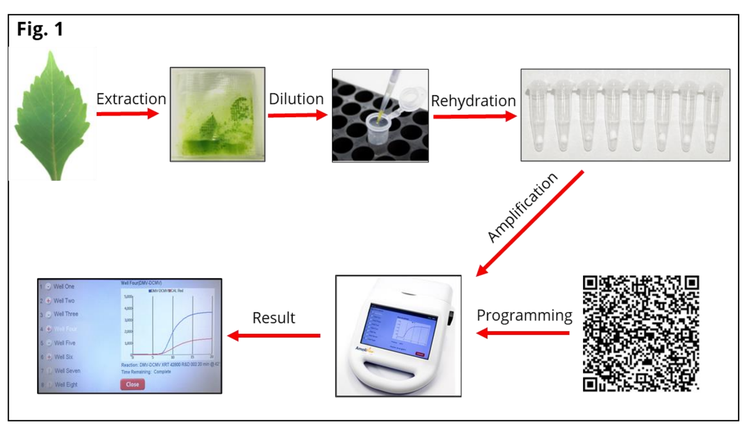

Figure 1. A workflow of the testing process for the DMV & DCMV AmplifyRP® XRT assay.

Results & discussion

1. Detection of DMV

DNA fragments containing the target region of various known DMV isolates were synthesized and tested in the presence of healthy dahlia extract. This assay detected all four DMV isolates (**Fig. 2A**). The DNA from the reference DMV isolate was serially diluted in 10 folds and further tested to determine analytical sensitivity. The assay detected as little as 10 ag (sample #6) of the DMV DNA fragment per reaction (**Fig. 2B**). The limit of detection (LOD) was determined to be 50 ag (or 71 copies) of the target DMV DNA per reaction. This is as sensitive as qPCR or LAMP (6) for DMV.

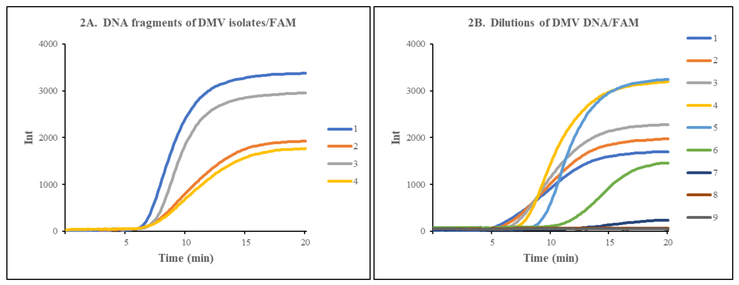

Figure 2. Detections of synthetic target DMV DNA fragments (**Fig. 2A**) and 10-fold dilutions of the DMV DNA fragment from the reference DMV isolate (**Fig. 2B**). **2A**, the DNA fragments from samples #1 to #4 represent the DMV isolates of Netherlands, Kentucky, Netherlands, and Portland (RefSeq), respectively. **2B**, amounts of the template DNA per reaction were 1.0 pg to 0.1 ag in 10-fold serial dilutions from samples #1 to #8, respectively. Sample #9 was healthy dahlia extract only.

2. Detection of DCMV

DNA fragments containing the target region of various known DCMV isolates were also synthesized and tested in the presence of healthy dahlia extract. The same assay detected all the DCMV isolates (**Fig. 3A**). The DNA from the DCMV New Zealand isolate was serially diluted in 10 folds and further tested to determine analytical sensitivity. This assay detected as little as 10 ag (sample #6) of the DCMV DNA fragment per reaction (**Fig. 3B**). The limit of detection (LOD) was determined to be 50 ag (or 66 copies) of the target DCMV DNA per reaction. This is as sensitive as qPCR (no LAMP assay available) for DCMV.

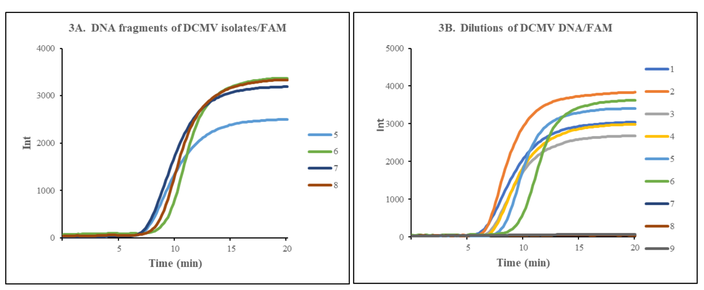

Figure 3. Detections of synthetic target DCMV DNA fragments (**Fig. 3A**) and 10-fold dilutions of the DCMV-NZ DNA fragment (**Fig. 3B**). **3A**, the DNA fragments from samples #5 to #8 represent the DCMV isolates of Beijing, New Zealand, Taiwan, and Netherlands, respectively. **3B**, amounts of the template DNA from the DCMV-New Zealand isolate were 1.0 pg to 0.1 ag per reaction in 10-fold serial dilutions from samples #1 to #8, respectively. Sample #9 was healthy dahlia extract only.

3. Detection of DMV, DCMV, and a control gene

This is a duplex assay capable of simultaneously detecting DMV, DCMV, and an endogenous plant gene as an internal control. Numerous dahlia samples were tested and the presence of DMV and/or DCMV were detected via this AmplifyRP® XRT assay (**Fig. 4A**) and confirmed by other molecular detection methods. A crude leaf extract (1:20 ratio of weight:GEB, considered to be 1x dilution) from a DMV & DCMV infected dahlia plant was also serially diluted in 10 folds and tested with this duplex assay. As shown in **Fig. 4B**, the assay detected down to 10^{-4}x dilution from this 1x extract (**Fig. 4B**). Meanwhile, it also detected the internal control from all the dahlia extracts – healthy or infected (**Fig. 4C**).

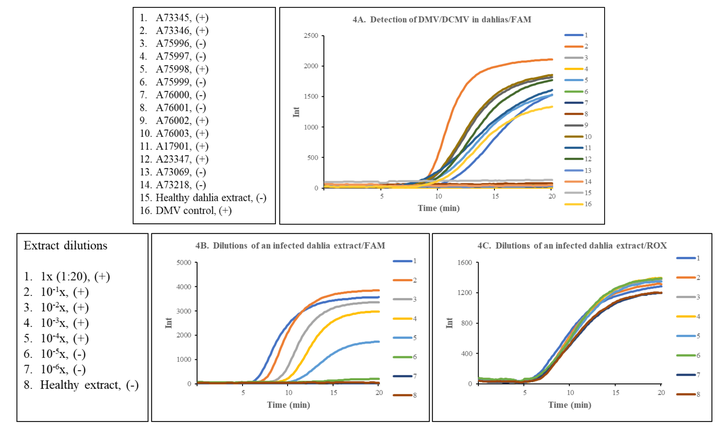

Figure 4. Simultaneous detections of DMV, DCMV, and an endogenous plant gene as a control. **4A**, detection of DMV and/or DCMV in dahlias. **4B**, Detection of DMV & DCMV from serially diluted extracts of a dahlia plant infected with DMV & DCMV. **4C**, detection of the control gene from the infected and healthy dahlia plants.

Conclusions

A duplex XRT assay of AmplifyRP® has been developed and commercialized for rapid and real-time detection of both DMV and DCMV. An endogenous plant gene as an internal control is also included and amplified simultaneously. The assay is specific to DMV and DCMV and does not cross-react with other dahlia-infecting pathogens. As shown in the **Table** below, the assay is also highly sensitive for detecting DMV and DCMV. This assay utilizes crude plant extracts as templates and runs at 42°C in a single PCR tube. The whole assay is completed in 20 minutes and applicable both in laboratories and in the field. This is the first report on the detection of both DMV and DCMV through RPA.

Virus	Analytical Sensitivity (LOD)	Diagnostic Sensitivity	Analytical Specificity	Diagnostic Specificity
DMV	71 copies per reaction	58/58 (100%)	Isolates from Germany, Netherlands, and USA	40/40 (100%)
DCMV	66 copies per reaction		Isolates from Beijing, Netherlands, New Zealand, and Taiwan	

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References

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