# National Diagnostic Protocol

*Potyvirus zeananus*

(Maize Dwarf Mosaic Virus; MDMV)

*NDP 52 V1*

This publication (and any material sourced from it) should be attributed as: Subcommittee on Plant Health Diagnostics (August 2024). National Diagnostic Protocol for Maize Dwarf Mosaic Virus – NDP52 V1. (Eds. Subcommittee on Plant Health Diagnostics) Authors Geering A, Thomas J, Persley D, Lovelock D. Reviewers Delmiglio C, Lovelock, D. ISBN 978-0-6481144-8-2 CC BY 3.0.

**Cataloguing data**
Subcommittee on Plant Health Diagnostics (August 2024). National Diagnostic Protocol for *Potyvirus zeananus* (Maize Dwarf Mosaic virus)– NDP52 V1. (Eds. Subcommittee on Plant Health Diagnostics) Authors Geering A, Thomas J, Persley D, Lovelock D.; Reviewers Delmiglio C, Lovelock, D. ISBN 978-0-6481144-8-2.

ISBN; 978-0-6481144-8-2

**Internet**
Report title is available at: https://www.plantbiosecuritydiagnostics.net.au/resources/?category=national-diagnostic-protocols

**Department of Agriculture, Fisheries and Forestry**

**Street Address:** Agriculture House, 70 Northbourne Avenue, Canberra 2601, Australia
**Postal Address:** GPO Box 858, Canberra City ACT 2601
**Switchboard Phone:** 02 6272 3933
**Web:** http://www.agriculture.gov.au

Inquiries regarding the licence and any use of this document should be sent to: copyright@aff.gov.au.

**Purpose**

National Diagnostic Protocols (NDPs) are diagnostic protocols for the unambiguous taxonomic identification of plant pests. NDPs:

- are a verified information resource for plant health diagnosticians
- are consistent with ISPM No. 27 – Diagnostic Protocols for Regulated Pests
- provide a nationally consistent approach to the identification of plant pests enabling transparency when comparing diagnostic results between laboratories; and,
- are endorsed by regulatory jurisdictions for use (either within their own facilities or when commissioning from others) in a pest incursion.

Where an International Plant Protection Convention (IPPC) diagnostic protocol exists it should be used in preference to NDPs, unless it is shown that the NDP has improved procedures for Australian conditions. NDPs may contain additional information to aid diagnosis. IPPC protocols are available on the IPPC website:

https://www.ippc.int/core-activities/standards-setting/ispms

**Process**

NDPs are facilitated and endorsed by the Subcommittee on Plant Health Diagnostics (SPHD). SPHD reports to Plant Health Committee and is Australia's peak technical and policy forum for plant health diagnostics.

NDPs are developed and endorsed according to Reference Standards developed and maintained by SPHD. Current Reference Standards are available at https://www.plantbiosecuritydiagnostics.net.au/work/subcommittee-on-plant-health-diagnostics/

NDPs are living documents. They are updated every 5 years or before this time if required (i.e. when new techniques become available).

**Document status**

This version of the National Diagnostic Protocol (NDP) for *xxx* is current as at the date contained in the version control box below.

| | |
|---|---|
| PEST STATUS | Not present in Australia |
| PROTOCOL NUMBER | NDP 52 |
| VERSION NUMBER | V1 |
| PROTOCOL STATUS | Endorsed |
| ISSUE DATE | August 2024 |
| REVIEW DATE | August 2029 |
| ISSUED BY | SPHD |

The most current version of this document is available from the SPHD website: https://www.plantbiosecuritydiagnostics.net.au/resources/?category=national-diagnostic-protocols

**Further information**

Inquiries regarding technical matters relating to this protocol should be sent to: sphd@aff.gov.au

# Contents

# 1 INTRODUCTION

*Potyvirus zeananus* (Maize dwarf mosaic virus (MDMV)) is a disease of maize and sorghum species. It belongs to a subgroup of closely related potyviruses that includes sorghum mosaic virus (SrMV), Johnsongrass mosaic virus (JGMV) and sugarcane mosaic virus (SCMV) (Kannan *et al.* 2018). JGMV and SCMV are already common and widespread in Australia and great care must be taken to distinguish these viruses from MDMV and SrMV, which are both exotic to Australia. There are no authenticated records of MDMV in Australia. Virus isolates collected by Taylor and Pares (1968) from northern NSW have erroneously been labelled MDMV and are almost certainly JGMV (Silva *et al*. 2016). Follow up diagnostic tests should be done for any potyvirus detected in maize or sorghum that is negative for JGMV as there is a strong possibility that the virus will be exotic (either MDMV or SrMV).

A method to distinguish these four viruses by Reverse transcription-polymerase chain reaction (RT-PCR) is presented. Finally, an alternative diagnostic method using enzyme-linked immunosorbent assay (ELISA) is presented.

## 1.1 Primary host range

In the field, MDMV has only ever been found infecting *Sorghum bicolor*, *Sorghum halepense*, *Sorghum sudanense* and *Zea mays* (Brunt *et al*. 1990; Rao *et al*. 1996; Toler 1985). The potential host range is, however, much broader. Experimentally susceptible hosts of MDMV include *Arundo donax*, *Bromus mollis*, *Bromo secalinus*, *Bromus tectorum*, *Chloris gayana*, *Cynodon dactylon*, *Echinochloa crus-galli*, *Eleusine coracana*, *Lagurus ovatus*, *Oryza sativa*, *Panicum acapillare*, *Panicum maximum*, *Panicum miliaceum*, *Paspalum dilatatum*, *Phalaris paradoxa*, *Rotboellia exaltata*, *Saccharum officinarum*, *Sacciolepsis indica*, *Setaria italica*, *Setaria viridis*, *Sorghum bicolor* and *Zea mays* (Brunt *et al*. 1990). Experimentally insusceptible hosts include *Anthoxanum odoratum*, *Avena sativa*, *Dactylis glomerata*, *Hordeum vulgare*, *Lolium perenne*, *Lolium temulentum*, *Poa pratensis*, *Secale cereale* and *Triticum aestivum* (Brunt *et al.* 1990).

# 2 TAXONOMIC INFORMATION

Realm *Riboviria*
Phylum *Pisuviricota*
Kingdom *Orthornavirae*
Class *Stelpaviricete*
Order *Patatavirales*
Family: *Potyviridae*
Genus: *Potyvirus*
Species*: Potyvirus zeananus*
Virus name: Maize dwarf mosaic virus

MDMV is a member of genus *Potyvirus* in the family *Potyviridae*. It has filamentous virions, 750 nm long × 13 nm wide, and an ssRNA genome consisting of 9515 nucleotides (Shukla *et al*. 1994). MDMV is closely related to SCMV, JGMV, SrMV and *Zea mosaic virus* (ZeMV) and together these constitute the sugarcane mosaic virus subgroup of potyviruses. In earlier literature, there was considerable confusion in the taxonomy of the sugarcane mosaic virus subgroup. Initially, all potyviruses infecting maize were labelled MDMV and all potyviruses infecting sugarcane were labelled SCMV. It is now recognised that both SCMV and MDMV infect maize causing similar mosaic symptoms, and what was previously referred to as MDMV strain B is in fact a strain of SCMV (Shukla *et al*. 1994). Five strains of MDMV, strains A, C, D, E and F, are still recognised based on symptomatology on inbred maize lines including inbred line N20 and frequency of transmission by different aphid species (Shukla *et al*. 1994; Kannan *et al.* 2018).

In pairwise sequence comparisons, the different species in the sugarcane mosaic virus subgroup have 69-92% amino acid sequence identity in the core of the coat protein (equivalent to $Asp_{70}$-$Arg_{285}$ in JGMV), and 59-83% amino acid sequence identity over the entire length of the coat protein (Seifers *et al*. 2000; Shukla *et al*. 1994). Amongst the different members of the subgroup, MDMV is most closely related to SrMV and most distantly related to JGMV (Seifers *et al*. 2000; Yang and Mirkov 1997). Bermuda grass southern mosaic virus (BgSMV) was proposed as a separate species because it naturally infects Bermuda grass (*Cynodon dactylon*) and goose grass (*Eleusine indica*), does not infect Johnsongrass (*Sorghum halepense*), it is not transmitted by *Rhopalosiphum maidis* and has a coat protein that contains a 90-nucleotide insertion (Zare *et al.* 2005; Mostafavi Neyshabouri *et al.* 2019). However, BgSMV does not meet the necessary demarcation criteria to define it as a separate species because it is serologically related to and shares 83.5-86.5% nucleotide and 91.1-92.3% amino acid sequence identity with MDMV.

# 3 DETECTION

## 3.1 Symptoms

Maize plants infected with MDMV show mosaic or mottle symptoms, particularly near the base of the youngest leaves (Figure 1). In hot weather, the mosaic symptoms may disappear and instead be replaced by general chlorosis in new growth. Severely infected plants are stunted, exhibit increased tillering and poor seed set (Figure 2 and 3). Infected plants are predisposed to other root rotting pathogens. Symptoms of MDMV in sorghum are similar to those in maize. Sorghum lines carrying the *rlf* gene develop a severe necrotic red leaf reaction when infected and grown under cool temperatures (Lapierre and Signoret 2004).

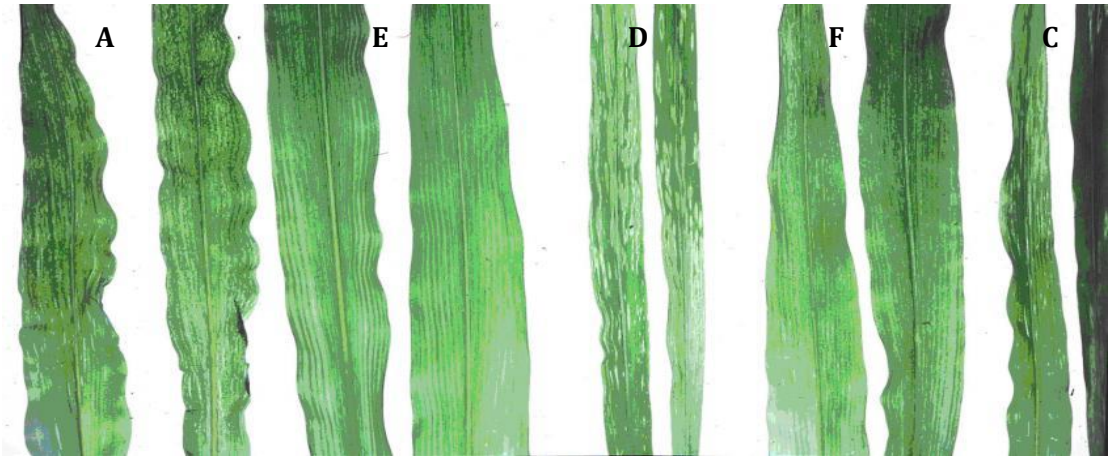


**Figure 1** Maize dwarf mosaic virus: symptoms on maize inbred line N20 caused by (left to right) strains A, E, D, F, and C. Photograph courtesy of Roy Gingery (USA).

**Figure 2** Maize dwarf mosaic virus: mosaic plus slight stunting of corn plant. Photograph courtesy of Roy Gingery (USA)

**Figure 3** Maize dwarf mosaic virus: mosaic symptoms on corn in southern Ohio. Photograph courtesy of Roy Gingery (USA)

### *3.1.1 Storage in the laboratory*

For short-term storage (less than 7 days), the sample should be stored at 4° C in a sealed plastic bag. For longer-term storage, the sample should be stored at -80° C.

## 3.2 Sampling

Symptomatic leaf material, particularly younger leaves, should be sampled with 10 leaves per bulk sample. Each bulk sample should be clearly labelled and double bagged to avoid the potential for cross-contamination.

# 4 IDENTIFICATION

MDMV can be detected by ELISA or RT-PCR. However, the ELISA test does have a mild-cross reaction with SCMV and JGMV, so any positive should always be confirmed by RT-PCR and/or sequencing. Reactions on indicator plants can be used as a guide for identification; however, any diagnosis should be supported by the other tests.

## 4.1 Characteristic reactions on indicator species

Host reactions may assist in the identification of exotic potyviruses in sorghum and maize. JGMV infection on the inbred sorghum line OKY8 produces a necrotic red stripe reaction, whereas this line is either symptomless or develops mosaic symptoms in response to infection with MDMV, SrMV and nearly all strains of SCMV (Persley *et al.* 1985; Tosic *et al.* 1990). Of the sugarcane mosaic virus subgroup of potyviruses, JGMV alone infects oats (Seifers *et al*. 2000; Tosic *et al*. 1990). The MDMV strain BgSMV does not infect Johnsongrass (Zare *et al.* 2005).

## 4.2 Serological tests – ELISA

The ELISA protocol for MDMV detects all known strains of MDMV but does have a mild-cross reaction with SCMV and JGMV. Positive material can be obtained from the ELISA supplier if appropriate permits are in place.

### *4.2.1 Equipment*

- 20, 100 and 1000 µL pipettes and tips
- Microcentrifuge and microcentrifuge tubes (2.0 mL)
- Small esky containing ice
- Mortar and pestle, or other mechanical disruption devices
- ELISA plates
- ELISA plate reader

### *4.2.2 Reagents*

- MDMV coating antibodies and enzyme conjugate (Catalogue no. 07059, Loewe Biochemica GmbH)
- Coating buffer
- PBS-T buffer
- ECL buffer (as Sample Buffer and Conjugate Buffer)
- PNP buffer
- Maximum of 0.3g of plant material

### *4.2.3 ELISA buffers*

***Coating buffer***

Dissolve in distilled water to 1000 mL:

| | |
|---|---|
| Sodium carbonate (anhydrous) | 1.59 g |
| Sodium bicarbonate | 2.93 g |

Adjust pH to 9.6 using 0.1 N acetic acid. Store at 4° C.

***PBS-T Buffer (Wash Buffer)***

Dissolve in distilled water to 1000 mL:

| | |
|---|---|
| Sodium chloride | 8.0 g |
| Sodium phosphate, dibasic (anhydrous) | 1.15 g |
| Potassium phosphate, monobasic (anhydrous) | 0.2 g |
| Potassium chloride | 0.2 g |
| Tween-20 | 0.5 g |

Adjust pH to 7.4. Store at 4° C.

***ECL Buffer (use as Sample Buffer and as Conjugate Buffer)***

Add to 1000 mL 1X PBST:

| | |
|---|---|
| Bovine serum albumin (BSA) | 2.0 g |
| Polyvinylpyrrolidone (PVP) MW 24-40,000 | 20.0 g |

Adjust pH to 7.4. Store at 4° C.

***PNP Buffer***

Dissolve in 800 mL distilled water:

| | |
|---|---|
| Magnesium chloride hexahydrate | 0.1 g |
| Sodium azide | 0.2 g |
| Diethanolamine | 97.0 mL |

Adjust pH to 9.8 with 0.1 N hydrochloric acid. Adjust final volume to 1000 mL with distilled water. Store at 4° C.

Note: Replace ELISA buffers if a precipitate or visible microbial growth is present in them.

### *4.2.4 ELISA Protocol and Interpretation of Results*

For MDMV detection using double-antibody sandwich (DAS)-ELISA with BIOREBA (Reinach, Switzerland), AGDIA (USA) or Loewe Biochemica (Germany), including interpretation of results, follow the manufacturer's instructions. All three ELISA's have mild cross reactions to SCMV and JGMV. The MDMV ELISA is known to cross-react with SrMV. The Loewe Biochemica ELISA has been validated in the verifying laboratory. **All ELISAs, regardless of whether they have been validated in another laboratory, should be validated in the testing laboratory before use.**

As the MDMV ELISA is known to cross-react with SrMV, **in the event of a positive ELISA result, the sample needs be tested by RT-PCR as per section 4.3.2.**

For further information:
http://www.bioreba.ch/saas/web/bioreba/web.aspx?PageID=58&search=MDMV
https://orders.agdia.com/agdia-set-mdmv-alkphos-sra-18000

## 4.3 Molecular methods

MDMV is closely related to SCMV and JGMV, both of which are common in Australia and are indistinguishable based on particle shape. MDMV can only be definitively identified by RT-PCR. **All RT-**

**PCRs, regardless of whether they have been validated in another laboratory, should be validated in the testing laboratory before use.**

Reagents

The tests were developed using the reagents below, however other general reagents (such as 1X TAE Buffer) and loading dyes can also be used.

***TBE (Tris/borate/EDTA) electrophoresis buffer***

| **Component** | **10´ stock solution:** | **5´ stock solution:** |
|---|---|---|
| Tris base | 108 g | 54 g |
| Boric acid | 55 g | 27.5 |
| 0.5 M EDTA pH 8.0 | 40 mL | 20 mL |

For TBE, a working solution of 0.5X provides more than enough buffering power, and almost all agarose gel electrophoresis is carried out using this buffer.

Store stock and working TBE solutions at room temperature.

***Gel loading buffers***

| **6X´ loading buffer** | **10X´ loading buffer** |
|---|---|
| 0.25% bromophenol blue | 0.25% bromophenol blue |
| 0.25% xylene cyanol FF | 0.25% xylene cyanol FF |
| 30% glycerol in water | 20% Ficoll 400 |
| store at 4° C | 0.1 M EDTA, pH 8.0 |
| | 1.0% SDS |
| | store at 4° C |

### *4.3.1 RT-PCR*

#### *Reagents*

- RNeasy® Plant Mini Kit (Qiagen) (or similar validated kit)
- OneStep RT-PCR Kit (Invitrogen® SuperScript™ One-Step RT-PCR with Platinum® Taq). (Alternatively, Qiagen or other RT-PCR kits validated within the laboratory can be used)
- Small esky containing ice
- 0.5X TBE
- Agarose
- 100 bp DNA marker
- Gel loading buffer
- DNA gel stain (e.g. SYBR Safe)
- Custom primers (see Table 1).

**Table 1** Primer sequences for the detection of maize dwarf mosaic virus (MDMV), Johnsongrass mosaic virus (JGMV), sugarcane mosaic virus (SCMV) and sorghum mosaic virus (SrMV) by RT-PCR.

| Target virus | Primer | Sequence (5'-3') | Size of amplicon (bp) | Reference |
|---|---|---|---|---|
| **MDMV** | MDMV-F1 | 5'-CAA CCA GGG CYG AAT TTG ATA G-3' | 336 | Geering, A. D. W. (Unpublished) |
| | MDMV-R1 | 5'-GTG CAA GGC TRA AGT CGG TTA-3' | | |
| **JGMV^** | JGMV-F1 | 5'-ATG GTR AGC AAC AAA GCC ATA C-3' | 367 | Geering, A. D. W. (Unpublished) |
| | JGMV-R1 | 5'-ACG GCT CAT CCA AAT TTC TCA T-3' | | |
| **SCMV^** | SCMV-F1 | 5'-TCT GGA CGG AAA TGT CGG C-3' | 253 | Geering, A. D. W. (Unpublished) |
| | SCMV-R1 | 5'-CCT GTR TCC TGC AGA CTG G-3' | | |
| **SrMV^** | SrMV-F1 | 5'-GCK GCA AAG AAG AAA GCT GAT G-3' | 442 | Geering, A. D. W. (Unpublished) |
| | SrMV-R1 | 5'-CAT CCA TCA TKG TCC AAA CAC C-3' | | |
| **MDMV*** | MDf3 | 5′-GAT GAG TTR AAY GTY TAT GCA CG AC- 3′ | 636 | Trzmiel and Jeżewska (2008) |
| | MDr1 | 5′-RTG CAT RAT TTG TCT GAA AGT TGG-3´ | | |
| **Potyvirus#** | Oligo1n | 5'-ATG GTH TGG TGY ATH GAR AAY GG-3' | 327 | Marie-Jeanne, *et al*., (2000) |
| | Oligo2n | 5'-TGC TGC KGC YTT CAT YTG-3' | | |
| **NADH** | AtropaNad 2.1a | 5'-GGA CTC CTG ACG TAT ACG AAG GAT C-3' | 188 | Thompson, *et al*., (2003) |
| | AtropaNad2.2b | 5'-AGC AAT GAG ATT CCC CAA TAT CAT-3' | | |

***** This primer set may pick up additional MDMV isolates but have only been verified by the reviewing laboratory (Delmiglio, 2014).

^Limitations of the SCMV, JGMV and SrMV RT-PCR may not be fully known until real-life samples are tested, however Bioinformatic analysis indicates that of the 165 or so full SCMV genomes published on GenBank, the SCMV primers will detect all of these isolates.

# This primer set is generic for potyviruses and has been designed to detect all known strains of MDMV and other potyviruses, with bioinformatic analysis indicating as such. This primer set should be used in addition to the specific MDMV primers. This primer set has, however, only been verified by the reviewing laboratory (Delmiglio, 2014).

**Table 2** Description of PCR controls to use.

| PCR controls | Description |
|---|---|
| Positive | Known MDMV/other relevant Potyvirus-infected sample (a positive control may be purchased from Loewe, Germany; DSMZ, Germany; BIOREBA, Switzerland; or alternatively use the target product cloned into a plasmid). |
| Negative | Healthy plant tissue (a negative control may be purchased from Loewe, Germany; DSMZ, Germany; BIOREBA, Switzerland). |
| No template control | Water |

### *4.3.2 Extraction of RNA*

RNA can be extracted using an RNeasy Plant Mini Kit (Qiagen) as per the manufacturer's instructions. Alternative extraction methods can be undertaken, provided they have been validated within the laboratory. Up to 500 mg of leaf material (either targeting symptomatic tissue or further away from the tip in asymptomatic tissue) can be ground, with buffer added at a ratio of 100 mg tissue : 1 mL buffer. See Table 2 for a description of the control material needed.

### *4.3.3 RT-PCR protocol using a OneStep RT-PCR mix (Invitrogen)*

One-step RT-PCR kit (Invitrogen® SuperScript™ III One-Step RT-PCR with Platinum® Taq, Catalogue No. 12574-026), is recommended, however already validated RT-PCR kits within individual laboratories (including two-step RT-PCR kit) can also be used. The PCR mix are shown in Table 3.

**Table 3** One-step RT-PCR conditions using SuperScript III One-Step RT-PCR System with Platinum *Taq* DNA Polymerase (Invitrogen) (Reaction volume: 25 µL).

| PCR reagents | Volume per reaction (25 µL) |
|---|---|
| Sterile distilled $H_2O$ | 8.0 |
| 2X master mix | 12.50 |
| 10 µM forward primer | 1.0 |
| 10 µM reverse primer | 1.0 |
| 10 mg/mL BSA (optional) | 1.25 |
| SuperScript III/Platinum Taq Mix | 0.50 |
| RNA template | 1.0 |

1. Into separate 0.2 mL PCR tubes, add 24 µL of reaction mix and 1 µL of RNA.
2. Place the reaction tubes in a thermal cycler and subject to:
    a. Geering (Unpublished): One cycle at 48° C for 45 min, followed by one cycle at 94° C for 2 min, 35 cycles at 95° C for 20 sec, 64° C for 20 sec and 72° C for 1 min and one cycle at 72° C for 5 min.
    b. Trzmiel, K. and Jeżewska, M. (2008): One cycle at 48° C for 45 min, followed by one cycle at 94° C for 2 min, 35 cycles at 94° C for 1 min, 60° C for 1 min and 72° C for 1 min and one cycle at 72° C for 5 min.

c. Marie-Jeanne, V., et al., (2000): One cycle at 48° C for 45 min, followed by one cycle at 94° C for 2 min, 40 cycles at 94° C for 30 sec, 50° C for 30 sec and 72° C for 1 min and one cycle at 72° C for 7 min.
d. Thompson et al., (2003): One cycle at 48° C for 45 min, followed by one cycle at 94° C for 2 min, 35 cycles at 94° C for 30 sec, 50° C for 30 sec and 72° C for 30 sec and one cycle at 72° C for 5 min.

3. Store RT-PCR products at 4° C.

### *4.3.4 Electrophoresis*

1. Determine size of gel and gel comb required.

| **Gel tray** | **Size** | **Volume of gel required (for 0.7 cm thickness)** |
|---|---|---|
| Small | 7.7 cm × 9.5 cm | 50 mL |
| Medium | 14.2 cm × 15.0 cm | 150 mL |
| Large | 18.3 cm × 25.0 cm | 300 mL |

2. Seal gel tray with adhesive tape and place on level base.
3. Place selected comb/s in grooves in tray.
4. Weigh agarose (1.5 g/100 mL of buffer) and place into suitable heat proof container.
5. Add required amount of gel running buffer (0.5 × TBE).
6. Boil until agarose is completely dissolved.
7. Allow to cool to approximately 50–55° C.
8. Add 10 µL of SYBR Safe per 100 mL of warm agarose.
9. Pour agarose into gel tray and allow to set.
10. Place gel in electrophoresis tank and submerge with 0.5 x TBE to a depth of at least 3 mm above the gel surface.
11. Add 2 µL of 6 x loading buffer per 10 µL of RT-PCR product and load into the wells of the gel.
12. Connect electrodes to powerpack and apply a voltage at 110 Volts for 30 min.
13. View gel on UV transilluminator, or a blue-light transilluminator. Record image using camera or gel documentation system.
14. The bands should be sufficiently resolved to allow easy distinction between the relatively similar sized bands of the different viruses, as per Figure 4.
15. Any positive results should be sent for Sanger sequencing (4.3.6).

***Results***

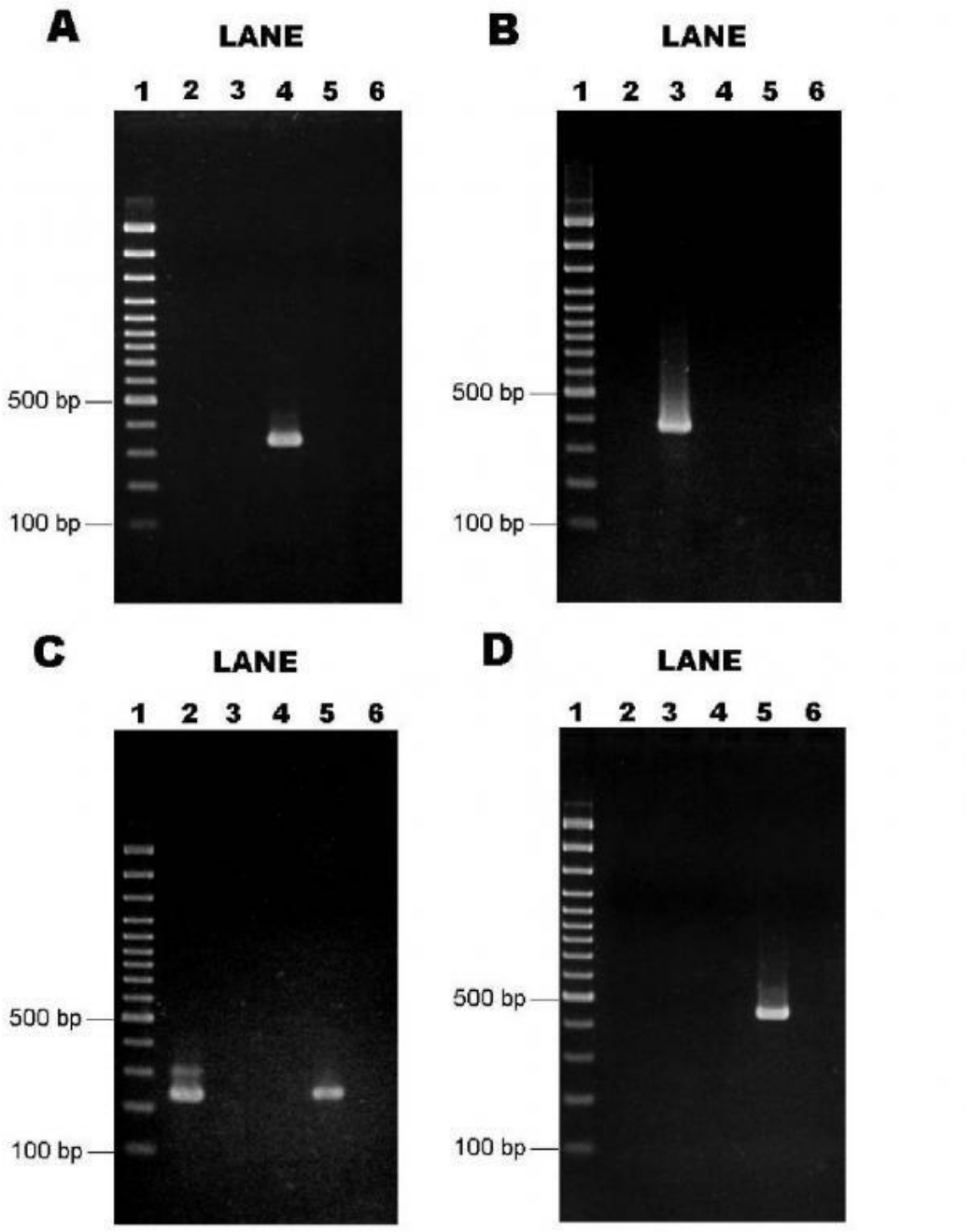


**Figure 4** Differentiation of the sugarcane mosaic virus subgroup of potyviruses by RT-PCR (Panel A, Maize dwarf mosaic virus (MDMV) assay*; Panel B, Johnsongrass mosaic virus (JGMV) assay; Panel C, Sugarcane mosaic virus (SCMV) assay; Panel D, Sorghum mosaic virus (SrMV) assay. Order of loading: Lane 1, 100 bp marker; Lane 2, SCMV; Lane 3, JGMV; Lane 4, MDMV; Lane 5, SrMV + SCMV.

*Amplified using the MDMV-F1/R1 primer set.

### 4.3.5 PCR Product clean-up and Sequencing

Following the manufacturer's instructions (QIAquick PCR Purification Kit, Qiagen or similar), it is recommended that the PCR products be cleaned directly from the RT-PCR tube or excised from the gel. The product along with the appropriate primers can then be sent to a sequencing facility (e.g. Micromon, Monash University) if it cannot be completed on site. The resulting sequences can then be compared to MDMV isolates on GenBank, including the type species (NC_003377 MDMV, NC_003398 SCMV, NC_003606 JGMV, NC_004035 SrMV).

### 4.3.6 Reference material

Dried leaf cultures of MDMV for use as positive controls are held in the Queensland Department of Agriculture and Fisheries Plant Virus Collection.

### 4.3.7 Guide to applying primer sequences for RT-PCR listed in table 1 for detection of MDMV

The following guide is recommended for using the primer sequences listed in Table 1 to detect MDMV virus and for a definitive interpretation of the results;

**1. Screening samples for MDMV**

a. Screen samples with both MDMV-specific RT_PCR primers (Table 1, Geering *et al.*, unpublished and Trzmiel and Jeżewska (2008)), as some MDMV isolates are not detected using one of the specific RT-PCR assays.

b. Simultaneously run the generic potyvirus RT_PCR assays to detect MDMV (Table 1, Marie-Jeanne, et al., (2000)), as the primer set for generic potyviruses are known to detect all known strains of MDMV.

**2. Screening samples for other viruses**

a. It is recommended that the samples are to be further screened for SCMV, SrMV and JGMV in the event of a negative result.

# 5 CONTACTS FOR FURTHER INFORMATION

Mr Denis Persley
Queensland Department of Agriculture and Fisheries
AgriScience Queensland, EcoSciences Precinct, Dutton Park, Queensland, Australia.

Dr Andrew Geering
Queensland Alliance for Agriculture and Food Innovation,
The University of Queensland, St Lucia, Queensland, Australia

Dr David Lovelock
Plant Health Australia
Level 1, 1 Phipps Close, Deakin ACT 2600
Phone: 02 6215 7700
Email: Dlovelock@phau.com.au

# 6 ACKNOWLEDGEMENTS

Prepared by Dr Andrew Geering, Dr John Thomas and Mr Denis Persley. The NDP was reviewed and verified by Dr Catia Delmiglio, Plant Health and Environment Laboratory (PHEL), Ministry for Primary Industries, Auckland-New Zealand. The NDP was further reviewed and updated by David Lovelock, Agriculture Victoria.

Photographs of MDMV-infected plants were generously provided by Roy Gingery.

# 7 REFERENCES

Berger PH, Zeyen RJ, Groth JV (1987) Aphid retention of maize dwarf mosaic virus (potyvirus): epidemiological implications. *Annals of Applied Biology* **111**: 337-344.

Brunt A, Crabtree K, Gibbs A (1990) *Viruses of tropical plants*. Wallingford, Oxon, UK: CAB International.

Delmiglio, C (2014) Peer Review and Verification Report of the SPHDS Diagnostic Protocol for *Maize dwarf mosaic virus* (MDMV). Plant Health and Environmental Laboratory, Ministry for Primary Industries.

Ford RE, Tosic M, Shukla DD (2004) Maize dwarf mosaic virus. *AAB Descriptions of Plant Viruses Online*, Description no. 341.

Kannan M, Ismail I, Bunawan H (2018) *Maize Dwarf Mosaic Virus*: From Genome to Disease Management. *Viruses* **10**: 492.

Lapierre H, Signoret PA (2004) *Viruses and virus diseases of Poaceae* (*Gramineae*). INRA Editions.

Marie-Jeanne V, Ioos R, Peyre J, Alliot B, Signoret P (2000). Differentiation of poaceae potyviruses by reverse transcription polymerase chain reaction and restriction analysis. *Journal of Phytopathology* **148:**141-151.

Mostafavi Neyshabouri FS, Yamchi A, Sabbagh SK, Masumi M (2019) Localization of genetic determinants for pathogenicity of Maize dwarf mosaic virus and Bermudagrass southern mosaic virus. bioRxiv; 651372. DOI: 10.1101/651372.

Persley DM, Henzell RG, Greber RS, Teakle DS, Toler RW (1985) Use of a set of differential sorghum inbred lines to compare isolates of sugarcane mosaic virus from Sorghum and maize in nine countries. *Plant Disease* **69**: 1046-1049.

Rao GP, Jain RK, Varma A (1996) Occurrence of maize dwarf mosaic virus on maize and sudangrass in India. *Plant Disease* **80**: 711.

Seifers DL, Salomon R, Marie-Jeanne V, Alliot B, Signoret P, Haber S, Loboda A, Ens W, She YM, Standing KG (2000) Characterization of a novel potyvirus isolated from maize in Israel. *Phytopathology* **90**: 505-513.

Shukla DD, Ward CW, Brunt AA (1994) *The Potyviridae*. CAB International, Wallingford, UK.

Silva, KN, Melo FL, Orilio AF, Nagata T, Silva MS, Fernandes CD, Fragoso RR, Dessaune SN, Resende RO (2016) Biological and molecular characterization of a highly divergent Johnsongrass mosaic virus isolate from *Pennisetum purpureum*. *Archives of virology* **161**: 1981-1986.

Taylor RH, Pares RD (1968) The relationship between sugar-cane mosaic virus and mosaic viruses of maize and johnson grass in Australia. *Australian Journal of Agricultural Science* **19**: 767-773.

Thompson JR, Wetzel S, Klerks MM, Vaskova D, Schoen CD, Spak J, Jelkmann W (2003) Multiplex RT-PCR detection of four aphid-borne strawberry viruses in *Fragaria* spp. in combination with a plant mRNA specific internal control. *Journal of Virological Methods* **111**: 85– 93.

Toler RW (1985) Maize dwarf mosaic, the most important virus disease of sorghum. *Plant Disease* **69**: 1011-1015.

Tosic M, Ford RE, Shukla DD, Jilka J (1990) Differentiation of sugarcane, maize dwarf, Johnsongrass, and sorghum mosaic viruses based on reactions of oat and some sorghum cultivars. *Plant Disease* **74**: 549-552.

Trzmiel K, Jeżewska M (2008) Identification of Maize dwarf mosaic virus in Maize in Poland. *Plant Disease* **92:** 981.

Yang ZN, Mirkov TE (1997) Sequence and relationships of sugarcane mosaic and sorghum mosaic virus strains and development of RT-PCR based RFLPs for strain discrimination. *Phytopathology* **87**: 932-939.

Zare A, Masumi M, Izadpanah K (2005) Bermuda grass southern mosaic virus: A distinct 321 potyvirus infecting several gramineous species in Iran. *Parasitica* **61**: 105-110.

Zeyen RJ, Stromberg EL, Kuehnast EL (1987) Long-range aphid transport hypothesis for maize dwarf mosaic virus: history and distribution in Minnesota, USA. *Annals of Applied Biology* **111**: 325-336.

# 8 APPENDICES

## 8.1 Biology

### *8.1.1 Stages of development*

MDMV is an obligate parasite and cannot survive outside of either its host or vector. *Sorghum halepense* (Johnsongrass), a perennial weed, is a critical overwintering host between annual corn and sorghum crops (Toler 1985). Even when frosts kill the foliage of *S. halepense*, the rhizomes of this plant persist and provide a refuge for the virus.

### *8.1.2 Transmission*

MDMV is transmitted in a non-persistent manner by a broad range of aphids including *Schizaphis graminum, Aphis maidiradicis, Aphis craccivora, Aphis fabae, Acyrthosiphon pisum, Myzus persicae, Aphis gossypii, Therioaphis maculata, Sitobion (Macrosiphum) avenae, Rhopalosiphum padi, Rhopalosiphum poae, Macrosiphum euphorbiae, Rhopalosiphum maidis, Brevicoryne brassicae* and *Rhopalosiphum fitchii* (Ford *et al*. 2004). Aphids can acquire and transmit the virus in a matter of minutes (Ford *et al.* 2004). Active spread of the virus often occurs with little evidence of aphid colonisation.

Non-persistently transmitted viruses are traditionally perceived as only being retained by the aphid for a maximum of 1–2 hours. However, under experimental conditions, viruliferous *Schizaphis graminum* have been observed to retain infectivity for over 20 hours (Berger *et al*. 1987). Epidemics of MDMV periodically occur in the northern states of the USA and even in Ontario in Canada, where the harsh winters prevent survival of the overwintering host of the virus, *Sorghum halepense* (Zeyen *et al*. 1987). It is believed that these epidemics are caused by large-scale migrations of air-borne aphids from more southerly latitudes in the USA. Immediately preceding an epidemic of MDMV in Minnesota in 1997, low-level jet winds swept through the Great Plains and it was estimated that with a wind assistance of 80 km/hr, aphids could have flown from Texas, more than 1500 km away, in as little as 20 hours (Zeyen *et al*. 1987).

Apart from aphid transmission, MDMV is also transmitted in dent corn seed at frequencies from 0.007% to 0.4% (Ford *et al*. 2004). Seed transmission of MDMV in sorghum is not recorded (Toler 1985), although this possibility cannot be discounted.

## 8.2 References

Berger PH, Zeyen RJ, Groth JV (1987) Aphid retention of maize dwarf mosaic virus (potyvirus): epidemiological implications. *Annals of Applied Biology* **111**: 337-344.

Ford RE, Tosic M, Shukla DD (2004) Maize dwarf mosaic virus. *AAB Descriptions of Plant Viruses Online*, Description no. 341.

Toler RW (1985) Maize dwarf mosaic, the most important virus disease of sorghum. *Plant Disease* **69**: 1011-1015.

Tosic M, Ford RE, Shukla DD, Jilka J (1990) Differentiation of sugarcane, maize dwarf, Johnsongrass, and sorghum mosaic viruses based on reactions of oat and some sorghum cultivars. *Plant Disease* **74**: 549-552.

Zeyen RJ, Stromberg EL, Kuehnast EL (1987) Long-range aphid transport hypothesis for maize dwarf mosaic virus: history and distribution in Minnesota, USA. *Annals of Applied Biology* **111**: 325-336.

# 9 DIAGNOSTICS PROCEDURES TO SUPPORT SURVEILLANCE

## 9.1 Introduction

*Potyvirus zeananus* (Maize dwarf mosaic virus; MDMV) is a disease of maize and sorghum and belongs to a subgroup of closely related potyviruses that includes sorghum mosaic virus (SrMV), Johnsongrass mosaic virus (JGMV) and sugarcane mosaic virus (SCMV) (Kannan *et al.* 2018). JGMV and SCMV are already common and widespread in Australia and great care must be taken to distinguish these viruses from MDMV and SrMV, both exotic to Australia, as symptoms can be similar in host plants.

Maize and sorghum plants infected with MDMV show mosaic or mottle symptoms, particularly near the base of the youngest leaves (Lapierre and Signoret 2004) (**Fig 1**).

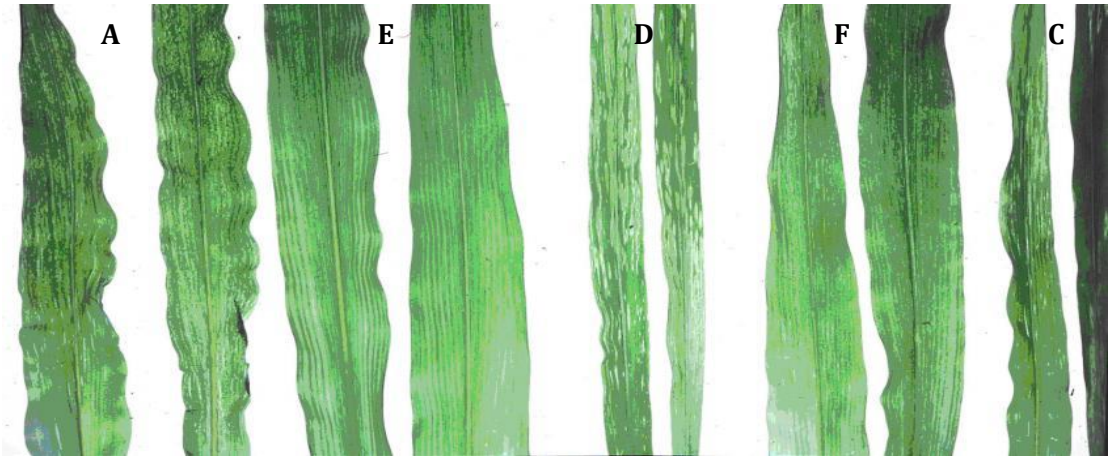


**Figure 1** Maize dwarf mosaic virus: symptoms on maize inbred line N20 caused by (left to right) strains A, E, D, F, and C. Photograph courtesy of Roy Gingery (USA).

Testing for MDMV includes common laboratory methods such as RT-PCR and ELISA, while an Agdia MDMV ImmunoStrip® can be used in both field and laboratory situations (Table 4). It is recommended that the laboratory undertake RT-PCR for identification, due to the mild cross reaction of SCMV and JGMV to the ELISA kits. Agdia's MDMV ImmunoStrip®, which according to the manufacturer are specific for MDMV and do not cross react with SCMV, provide results within 5-10 minutes as stated by the manufacturer.

**Table 4:** Diagnostic protocols for the identification of Maize dwarf mosaic virus.

| Method | Identification level | Identification Confidence | Deployment (Field/Lab) | Required Time | Throughput (No. of samples) |
|---|---|---|---|---|---|
| **Characteristic reactions on indicator species (4.1)** | To Genus | Low (<90%) | Glasshouse/Laboratory | >1 w | Medium (25-50) |
| **RT-PCR (4.3)** | To Species/Strain | High (99%+) | Laboratory | <1 w | High (100 s) |
| **ELISA (4.2)** | To Genus | Low (<90%) (Cross reacts with SCMV & JGMV) | Laboratory | <1 w | Very High (1000 s) |
| **Agdia Immunostrip (9.3)** | To Species | Medium (90-99%) | Field/Laboratory | <1 h | Medium (25-50) |

## 9.2 Sampling

Symptomatic leaf material, particularly younger leaves, should be sampled with 10 leaves per bulk sample. Each bulk sample should be clearly labelled and double bagged to avoid the potential for cross-contamination.

## 9.3 In-field Testing

In-field testing will potentially help surveillance teams identify suspect plants. It will also help with laboratory workloads as it will potentially minimise the number of samples submitted for testing. Agdia's MDMV ImmunoStrip® is a lateral flow device suited for in-field testing (instructions are supplied with the kit). The test does not cross react with other pathogens that may infect Poaceae species.

More information can be found on the Agdia website (https://orders.agdia.com/agdia-immunostrip-for-mdmv-isk-18000).

## 9.4 Laboratory Tests

Laboratory methods for the detection of MDMV include enzyme-linked immunosorbent assay (ELISA), with positives to be confirmed by RT-PCR, and are located in sections 4.2 and 4.3 respectively.

## 9.5 Acknowledgements

This surveillance section was prepared by David Lovelock and checked by Fiona Constable, Agriculture Victoria. Photographs of MDMV-infected plants were generously provided by Roy Gingery.

## 9.6 References

Kannan M, Ismail I, Bunawan H (2018) Maize Dwarf Mosaic Virus: From Genome to Disease Management. *Viruses* **10**: 492.

Lapierre H, Signoret PA (2004) *Viruses and virus diseases of Poaceae (Gramineae).* INRA Editions.