Natural infection of *Cucumber mosaic virus*, *Pea seed-borne mosaic virus* and *Turnip yellows virus* in a fenugreek crop (*Trigonella foenum-graecum*)

Mohammad Aftab¹ · Narelle Nancarrow¹ · Angela Freeman² · Jenny Davidson³ · Brendan Rodoni² · Piotr Trębicki¹

Received: 18 October 2017 / Accepted: 22 December 2017 / Published online: 6 January 2018
© Australasian Plant Pathology Society Inc. 2018

Abstract

For the first time, *Cucumber mosaic virus* (CMV), *Pea seed-borne mosaic virus* (PSbMV) and *Turnip yellows virus* (TuYV) were detected in a naturally infected fenugreek (*Trigonella foenum-graecum*) crop in Australia. The level of infection reached an alarming 90%, 75% and 20% respectively for CMV, TuYV and PSbMV, resulting in total crop loss. Both CMV and TuYV were detected in non-symptomatic fenugreek plants while PSbMV was not. Seed lots from the crop in question were established, planted in the glasshouse and tested for a range of seed-borne viruses, none of which were detected. Additionally, fenugreek seedlings grown from the seeds in the glasshouse were found to be readily infected with a TuYV isolate from canola when inoculated using viruliferous *Myzus persicae*.

Keywords Virus symptoms · Crop loss · CMV · PSbMV · TuYV · BWYV

Fenugreek (*Trigonella foenum-graecum*) is a multi-purpose, self-pollinated, annual, herbaceous legume crop (family Fabaceae). It is cultivated worldwide, its leaves are used as a vegetable or as a herb and seeds are used as spice. Fenugreek has been grown in Australia as a minor crop since the mid-1980s mainly for seed production and green manure (McCormick et al. 2006). Fenugreek has been reported as a natural host for *Tobacco streak virus* (TSV) (Kaiser et al. 1991), *Soybean dwarf virus* (SbDV), *Faba bean necrotic yellows virus* (FBNYV) and *Beet western yellows virus* (BWYV) (Bekele et al. 2005) and as an experimental host for a number of viruses including *Alfalfa mosaic virus* (AMV) (Latham and Jones 2001), *Bean yellow mosaic virus* (BYMV) (Barnett and Zeyong 1982), CMV (Iqbal et al. 2011), *Faba bean necrotic yellows virus* (FBNYV) (Franz et al. 1997),

Turnip mosaic virus (TuMV) (Fisher and Lockhart 1976) and *Pea enation mosaic virus* (PEMV).

A disease outbreak in fenugreek (cv. Sungold) in Mundoora, South Australia was inspected in mid-October 2016, the average temperature for that region for October 2016 was 21.2 °C (Station 21,133, www.bom.gov.au). Samples were collected and tested for the presence of a range of viruses. Although the majority (approximately 90% of the fenugreek crop) of plants were dying or dead (Fig. 1h), four types of virus-like symptoms were recorded: (i) non-symptomatic plants (Fig. 1a, d), (ii) red discolouration (Fig. 1b, e), (iii) mild red discolouration (Fig. 1f) and (iv) yellow discolouration (Fig. 1c, g). Tip necrosis was associated with red and yellow leaf discolouration. To identify the causative agent associated with the symptoms and crop loss, one hundred samples were randomly collected and tested for AMV, BYMV, CMV, PSbMV, *Bean leafroll virus* (BLRV), TuMV and TuYV (with a broad-spectrum antisera of BWYV) using tissue blot immunoassay (TBIA) (Fig. 2) as described by Freeman et al. (2013). CMV, TuYV and PSbMV were detected in 90%, 75%, and 20% of samples, respectively. AMV, BYMV, BLRV and TuMV were not detected. Dry leaf samples of fenugreek were lodged with the Victorian Plant Pathogen Herbarium (VPRI) and assigned the numbers VPRI 42919a CMV, VPRI 42919b PSbMV and VPRI 42919c TuYV.

✉ Mohammad Aftab
mohammad.aftab@ecodev.vic.gov.au

¹ Agriculture Victoria, Horsham Centre, 110 Natimuk Rd, Horsham, VIC 3400, Australia

² Agriculture Victoria, AgriBio, Centre for AgriBioscience, La Trobe University, 5 Ring Road, Bundoora, VIC 3083, Australia

³ South Australian Research and Development Institute, GPO Box 397, Adelaide 5001, Australia

Fig. 1 Illustration of different symptoms recorded in a naturally infected fenugreek crop with a range of host species; **a** non-symptomatic fenugreek shoot, **b** fenugreek with red discolouration, **c** fenugreek with yellow discolouration, **d** non-symptomatic fenugreek leaves, **e** severe red discolouration, **f** mild red discolouration, **g** yellow discolouration, **h** over 90% of the infected fenugreek crop with prominent red discolouration, the obvious green plants are common sowthistle with a healthy chick-pea crop on the left

Selected samples that tested positive for CMV, PSbMV and TuYV with TBIA were also tested by RT-PCR to confirm virus identities. Total RNA was extracted from leaf tissue using an RNeasy Plant Mini Kit (Qiagen) with a modified

lysis buffer (MacKenzie et al. 1997; Constable et al. 2007). Conventional RT-PCR was performed using published primers and PCR cycling conditions for CMV, PSbMV, TuYV and BWYV (Table 1). The SuperScript III One-Step

Fig. 2 Cross sections of bundles of fenugreek stems blotted onto nitrocellulose membranes and processed for **a** CMV, **b** PSbMV and **c** TuYV. Dark blue/purple discolouration represents virus infection. Each bundle has been marked with the number of positives designated for that particular bundle

Table 1 Sequence, target gene and expected PCR product size for primers used for RT-PCR

Primer name	Primer sequence (5'-3')	Target gene	Expected product size (bp)	Reference
CMV1	TATGATAAGAAGCTTGTTCGCGCA	Coat protein (RNA3)	500	Bariana et al. 1994
CMV2	TTTTAGCCGTAAGCTGGATGGACAACCC			
PSBCP1	AATGGCGCATTTTCAGTGACG	Coat protein	235	Freeman et al. 2013
PSBCP2	CCCYTCCAAGCCAAATAGGC			
TuYVorf0F	ACAAAAGAAACCAGGAGGGAATCCTTA	P0	780	Wilson et al. 2012
TuYVorf0R	TCATACAAACATTTTCGGTGTAGAC			
BWYV cpF	CAGTAGCCGGTATTACTTAGTCTACC	Coat protein	648	Wilson et al. 2012
BWYV cpR	GGCACTTCATAGTGATTCTAAAAGAA			

RT-PCR System (Invitrogen) was used for RT-PCR according to the manufacturer's instructions except the total reaction volume was 25 µl and the reverse transcription step was 48 °C for 45 min. PCR products of the expected sizes (Table 1) were amplified for CMV, PSbMV and TuYV while samples were not positive for BWYV. CMV, PSbMV and TuYV amplicons were purified using a PCR Purification Kit (Qiagen) according to the manufacturer's instructions and directly sequenced (Australian Genome Research Facility, Melbourne). Nucleotide sequences were edited using ChromasLite v2.01 (Technelysium) and compared to the nucleotide collection database using NCBI BLASTn comparisons (NCBI 1988) with default parameters to obtain nucleotide identities. Nucleotide sequences were aligned using MUSCLE (Edgar 2004) and Maximum Likelihood trees were constructed using the Kimura 2-parameter model with a discrete Gamma distribution and 1000 bootstrap replicates in MEGA 7 (Kumar et al. 2016). Sequences were deposited in GenBank (Clark et al. 2016) with accession numbers MF176650 (CMV Fg-SA), MF176651 (PSbMV Fg-SA) and MF176652 (TuYV Fg-SA).

The 489 bp partial coat protein nucleotide sequence from the CMV isolate from fenugreek showed 99% nucleotide identity to subgroup II CMV isolates from the United States (JX227938) and Australia (M21464) while only showing 77–79% nucleotide identity to subgroup IA (D10538 and U66094) and subgroup IB (L36525 and AB008777) isolates. Additionally, the sequence contained an EcoRI restriction site typical of CMV subgroup II isolates (Wylie et al. 1993) and phylogenetic analysis of the partial coat protein sequence (Fig. 3a) shows the isolate from fenugreek is more closely related to subgroup II isolates than subgroup IA and IB isolates. The 225 bp partial coat protein nucleotide sequence from the PSbMV isolate from fenugreek showed 100% nucleotide identity to Australian pathotype P-4 isolates (AH011199, HQ185578) from pea hosts (Wylie et al. 2011), but 95–96% identity to pathotype P-1 (D10930) and pathotype P-2 (AJ252242) isolates. Phylogenetic analysis of the coat protein sequence (Fig. 3b) also groups the isolate from fenugreek with pathotype P-4 isolates rather than pathotype P-1 and P-2

isolates. The 711 bp partial P0 nucleotide sequence from the TuYV isolate from fenugreek showed 99% nucleotide identity to a number of Australian TuYV isolates (HQ543127, HQ543129, HQ543132) from pea and broccoli (*Brassica oleracea* var. *italica*) hosts (Wilson et al. 2012) while only showing 89–90% nucleotide identity to other TuYV isolates, including two Australian isolates (HQ543119 and JQ862472). Phylogenetic analysis of the P0 sequence (Fig. 3c) also shows that the isolate from fenugreek is more closely related to some TuYV isolates than others as the isolates fall into two groups, and isolates in both groups are distinct from BWYV.

A mixture of symptomatic and non-symptomatic fenugreek, self-sown lentil (*Lens culinaris*), self-sown field pea (*Pisum sativum*), burr medic (*Medicago polymorpha*) and barrel medic (*Medicago truncatula*) plants were collected from the same fenugreek field and tested using TBIA for the viruses mentioned above as well as for *Subterranean clover stunt virus* (SCSV) and FBNYV. CMV, PSbMV and TuYV were all detected however PSbMV was not found in burr medic or barrel medic (Table 2). All self-sown lentil and burr medic plants tested were positive for CMV while barrel medic had 60% CMV infection. Surprisingly, test results of non-symptomatic plants revealed reasonably high levels of infection. For example, all 3 self-sown field pea plants were positive for CMV, PSbMV and TuYV, despite two of the three plants displaying no symptoms. Although the percent of virus infection was higher in symptomatic fenugreek plants with red and yellow discolouration than in non-symptomatic plants (Table 2), non-symptomatic fenugreek plants still had 25% CMV and 13% TuYV infection. PSbMV was not detected in non-symptomatic fenugreek or in fenugreek and medics showing red discolouration. All field pea plants showing yellow discolouration that were tested were infected with PSbMV but infection in fenugreek and lentil with yellow discolouration was 25% and 60%, respectively. TuYV was detected in fenugreek with all symptom types and was also found in all other plant species tested, the percent infection in field pea was highest followed by fenugreek, medics and lentil (Table 2). AMV, BYMV, BLRV, TuMV, FBNYV and SCSV were not found in any of these diagnostic plant samples (Table 2).

Fig. 3 Phylogenetic relationships of **a** CMV partial coat protein, **b** PSbMV partial coat protein and **c** TuYV partial P0 nucleotide sequences of virus isolates from fenugreek (CMV MF176650, PSbMV MF176651 and TuYV MF176652) with other isolates of CMV, PSbMV and TuYV, respectively. GenBank accession numbers and isolate names are shown on the trees

a CMV

b PSbMV

c TuYV

Transmission of TuYV from canola to fenugreek var. Sungold (the seeds from which the infected crop was established) was assessed in a glasshouse experiment. 133 seedlings grown in insect proof cages were inoculated two weeks after germination with a known TuYV isolate using viruliferous *Myzus persicae*. Additional non-inoculated plants

were kept as a negative control. Four weeks post inoculation, plants were tested for TuYV using TBIA, 84% of inoculated plants were positive, non-inoculated control plants were negative and non-symptomatic. To assess seed health, the fenugreek seeds were also tested for a range of viruses. One hundred seeds were sown in insect-proof cages in the glasshouse.

Table 2 Percent of each virus species in symptomatic and non-symptomatic plants collected from a fenugreek crop naturally infected with virus and tested for a range of viruses using tissue blot immunoassay (TBIA). Plants are grouped according to visual symptoms

Type of sample	Visual symptoms	Percent virus infection			
		CMV	PSbMV	BWYV (TuYV)	AMV BYMV BLRV FBYV SCSV TuMV
Fenugreek	Non-symptomatic	25	0	13	0
Fenugreek	Mild red discolouration	67	0	67	0
Fenugreek	Red discolouration, tip necrosis	86	0	79	0
Fenugreek	Yellow discolouration, tip necrosis	100	25	50	0
Self-sown lentil	Yellow discolouration	100	60	40	0
Self-sown field pea	Non-symptomatic	100	100	100	0
Self-sown field pea	Yellow discolouration	100	100	100	0
Burr medic	Red and yellow discolouration	100	0	67	0
Barrel medic	Red and yellow discolouration	60	0	60	0

Four weeks later, seedlings were tested for the presence of AMV, BYMV, CMV and PSbMV using TBIA. All seedlings tested for these seed-borne viruses were negative.

This is a first record of natural infection of CMV, PSbMV and TuYV in a fenugreek crop in Australia. Previously, fenugreek has been reported as an experimental host of CMV (Iqbal et al. 2011) and a natural host of BWYV (Bekele et al. 2005) but has not been reported as a natural host of CMV, PSbMV or TuYV. At present, no antibodies are available to capture TuYV so a broad-spectrum BWYV antiserum was used to detect TuYV. PCR testing using specific TuYV and BWYV primers and direct sequencing of the TuYV PCR products confirmed that the samples that tested positive for BWYV using TBIA were TuYV. A similar result was found by Wilson et al. (2012) during a survey of vegetable crops in Tasmania, Australia where serological testing indicated the presence of BWYV but found that TuYV was being detected by the BWYV antisera. In the randomly collected diagnostic samples, the incidence of CMV and TuYV was very high. In the mixed infection of CMV, PSbMV and TuYV, the dominant field symptoms were red discolouration of whole plants however some plants had yellow leaves and a few plants were non-symptomatic. It was previously suggested that yellow discolouration is due to CMV infection in medics (Freeman and Aftab 2011). Most infected plants had mixed infections so it was difficult to establish a link between the presence of a particular virus species and symptom type (Table 2). Plants with only yellow but no red discolouration were found to be infected with a mixture of all three viruses (CMV, PSbMV, TuYV) however, plants with mild red, severe red or yellow discolouration, along with some non-symptomatic plants, were found to be infected with CMV and TuYV. The fenugreek plants inoculated with TuYV in the glasshouse experiment showed similar symptoms to the naturally infected field plants, suggesting the link between TuYV infection and symptom expression.

Fenugreek has an advantage over other pulse crops as it is less susceptible to fungal diseases, although it is susceptible to bacterial blight (McCormick and Hollaway 1999). We did not notice any visible fungal and bacterial symptoms so the fenugreek plants were not tested for fungal and bacterial diseases.

Acknowledgements This research was funded by Grains Research and Development Corporation and Department of Economic Development, Jobs, Transport and Resources Victoria. We thank Mrs. Marzena Krysinska-Kaczmarek SARDI for initial fenugreek blots, Linda Zheng for assistance with phylogenetic analyses and Jacky Edwards and Robyn Brett for assistance lodging the dried leaf sample in the Victorian Plant Pathogen Herbarium.

References

- Bariana HS, Shannon AL, Chu PWG, Waterhouse PM (1994) Detection of five seed-borne legume viruses in one sensitive multiplex polymerase chain reaction test. *Phytopathology* 84:1201–1205
- Barnett OW, Zeyong XU (1982) *Bean yellow mosaic virus*. In “plant viruses online: descriptions and lists from the VIDE database”
- Bekele B, Kumari S, Ali K, Yusaf A, Makkouk K, Aslake M, Ayalew M, Girma G, Hailu D (2005) Survey of viruses affecting legume crops in the Amhara and Oromia regions of Ethiopia. *Phytopath Medit* 44: 235–246
- Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW (2016) GenBank. *Nucleic Acids Res* 44(D1):D67–D72
- Constable FE, Joyce PA, Rodoni BC (2007) A survey of key Australian pome fruit growing districts for exotic and endemic pathogens. *Austr Plant Pathol* 36:165–172
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32(5):1792–1797
- Fisher HV, Lockhart BEL (1976) A Moroccan isolate of *Turnip mosaic virus* infection to garden pea and other legumes. *Plant Dis Rep* 60: 398–401
- Franz A, Makkouk KM, Vetten HJ (1997) Host range of *Faba bean necrotic yellows virus* and potential yield loss in infected faba bean. *Phytopath Medit* 36:94–103
- Freeman A, Aftab M (2011) Effective management of viruses in pulse crops in south eastern Australia should include management of weeds. *Austr Plant Pathol* 40:430–441

- Freeman A, Spackman M, Aftab M, McQueen V, King S, van Leur J, Loh MH, Rodoni B (2013) Comparison of tissue blot immunoassay and reverse transcription polymerase chain reaction assay for virus-testing pulse crops from a south-eastern Australia survey. *Austr Plant Pathol* 42:675–683
- Iqbal S, Ashfaq M, Shah H (2011) Biological characterisation of Pakistani isolates of *Cucumber mosaic virus*. *Pak J Bot* 43:3041–3047
- Kaiser WJ, Wyatt SD, Klein RE (1991) Epidemiology and seed transmission of two *Tobacco streak virus* pathotypes associated with seed increases of legume germ plasm in eastern Washington. *Plant Dis* 75:258–264
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33(7):1870–1874
- Latham LJ, Jones RAC (2001) Incidence of virus infection in experimental plots, commercial crops and seed stocks of cool season crop legumes. *Austr J Agric Res* 52:397–413
- MacKenzie DJ, McLean MA, Mukerji S, Green M (1997) Improved RNA extraction from woody plants for the detection of viral pathogens by reverse transcription-polymerase chain reaction. *Plant Dis* 81:222–226
- McCormick KM, Hollaway GJ (1999) First report of bacterial blight in fenugreek (*Trigonella foenum-graecum*) caused by *Pseudomonas syringae* pv. *syringae*. *Austr Plant Pathol* 28:338
- McCormick K, Norton R, Eagles HA (2006) Fenugreek has a role in south-eastern Australian farming systems. Proceedings of the 13th Australian agronomy conference. 10–14 September 2006 Perth, Western Australia. Pp 1–7
- National Center for Biotechnology Information (NCBI) [Internet] (1988) Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. Available from: <https://www.ncbi.nlm.nih.gov/>. Accessed Nov 2017
- Wilson CR, Lambert SJ, Dann AL, Cross P, Hay FS (2012) Occurrence of viruses within Tasmanian vegetable crops and identification of a novel *Polevirus* infecting pea. *Austr Plant Pathol* 41:311–319
- Wylie S, Wilson CR, Jones RAC, Jones MGK (1993) A polymerase chain reaction assay for *Cucumber mosaic virus* in lupin seeds. *Austr J Agric Res* 44:41–51
- Wylie SJ, Coutts BA, Jones RAC (2011) Genetic variability of the coat protein sequence of pea seed-borne mosaic virus isolates and the current relationship between phylogenetic placement and resistance groups. *Arch Virol* 156(7):1287–1290