Journal of General Plant Pathology (2020) 86:227–232
https://doi.org/10.1007/s10327-020-00912-8

DISEASE NOTE

First report of leaf curling and yellowing caused by ageratum yellow vein virus in *Phaseolus vulgaris* in Okinawa Prefecture, Japan

Yasuhiro Tomitaka[1] · Ayako Yamaguchi[3] · Sayumi Tanaka[1] · Shinji Kawano[2] · Yuna Tamayose[3] · Masatoshi Onuki[1]

Received: 23 April 2019 / Accepted: 24 November 2019 / Published online: 6 March 2020
© The Phytopathological Society of Japan and Springer Japan KK, part of Springer Nature 2020

Abstract
In November 2016, leaf curling and yellowing were observed in common bean plants on Ishigaki Island, Okinawa Prefecture, Japan. Those symptoms suggested a virus-like disease. PCR using specific primers for ageratum yellow vein virus (AYVV) yielded PCR products of the expected size from diseased plants. The complete nucleotide sequence of the DNA-A shared 99.9% identity with that of the AYVV-Ishigaki isolate. Further, the virus was transmitted by *Bemisia tabaci* Middle East-Asia Minor 1 and Mediterranean. This is the first report of the natural occurrence of AYVV in common bean plants.

Keywords Ageratum yellow vein virus · *Phaseolus vulgarism* · *Bemisia tabaci*

Common bean, *Phaseolus vulgaris*, is commercially important in Okinawa Prefecture, Japan. It can be cultivated in unheated greenhouses during winter in subtropical Okinawa. In November 2016, leaf curling and yellowing were observed in commercial beans (cv. Bikkuri Jumbo) on Ishigaki Island, Okinawa, Japan. Symptoms were first observed on a few plants near the entrance of the greenhouse before they were found on other plants during the cultivation period. This pattern suggested that the causative agent was spread by an insect-transmitted virus. The aim of the present study was to identify the pathogen.

Three common bean plants with typical leaf curling and yellowing (Fig. 1a) were collected from the field. All samples were stored at − 80 °C until use in the analysis described below. The symptoms suggested that the disease was caused by infection with a virus such as begomovirus. Since ageratum yellow vein virus (AYVV) and tomato yellow leaf curl virus (TYLCV) are major begomoviruses infecting crops and weeds in Okinawa Prefecture (Andou et al. 2010; Ueda et al. 2009), we conducted PCR with specific primers for AYVV and TYLCV (AYmulti-V and AYmulti-C for AYVV, TYIL-V3 and TYCON-C for TYLCV-Israel [IL] strain, TYMld-V3 and TYCON-C for TYLCV-Mild [Mld] strain) (Table 1). Total DNA was extracted from the leaves showing yellow leaf curl symptoms using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The concentration of the extracts was measured using a NanoDrop 2000 (Thermo Fisher Scientific, Witham, MA, USA). PCR was performed with Ex Taq DNA polymerase (TaKaRa Bio, Kusatsu, Japan) using the extracted DNA (100 ng) as a template. Total DNA extracted from AYVV-Ishigaki isolate (GenBank accession AB306314), TYLCV-IL isolate, or TYLCV-Mld isolate infected tomato plants were used for this experiment as positive control. The following conditions were used for PCR: 98 °C for 2 min followed by 35 cycles of 10 s at 98 °C, 30 s at 53°C, and 2 min at 72 °C. PCR products were then separated by 2% agarose gel electrophoresis. Amplicons of the expected size (approximately 200 bp) from all affected common bean samples and AYVV positive control were observed in the AYVV lanes, whereas there were no amplicons of DNA-A in the TYLCV-IL or TYLCV-Mld lanes in any common bean sample (Fig. 2).

To detect DNA-A, DNA-β, and DNA-α, we also conducted PCR with universal primers (V/C for DNA-A, Beta01/Beta02 for DNA-β, DNA101/DNA102 for DNA-α) of begomoviruses, previously reported (Briddon et al. 2002; Briddon and Markham 1994; Bull et al. 2003) (Table 1).

✉ Yasuhiro Tomitaka
yasut@affrc.go.jp

[1] NARO Kyushu Okinawa Agricultural Research Center, 2421 Suya, Koshi, Kumamoto 861-1192, Japan

[2] Okinawa Prefectural Agricultural Research Center, 820 Makabe, Itoman-City, Okinawa 901-0336, Japan

[3] Okinawa Prefectural Plant Protection Center, 123 Maji, Naha-City, Okinawa 902-0072, Japan

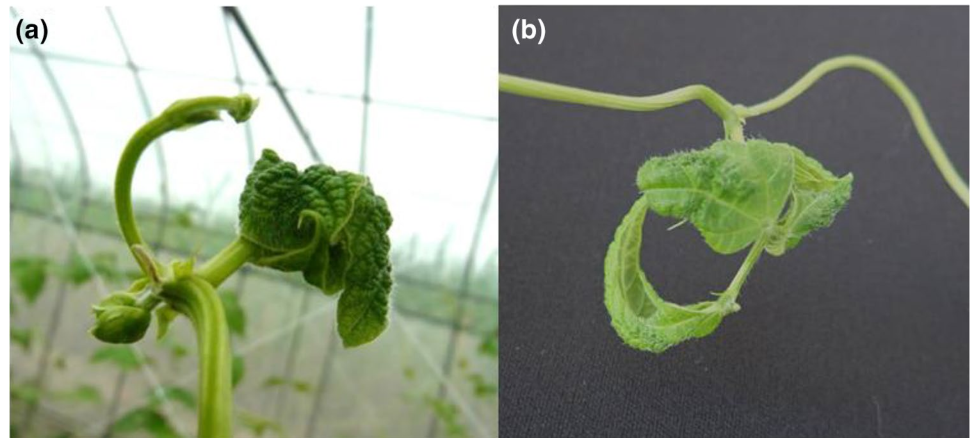

Fig. 1 Leaf curling and yellowing on *Phaseolus vulgaris* cv. Bikkuri Jumbo infected with ageratum yellow vein virus in the field (**a**) and in a transmission test (**b**)

Total DNAs of AYVV-Ishigaki isolate and common bean samples as described above were used in the PCR analyses. In addition, the complete nucleotide sequence of DNA-α of AYVV-Pakistan isolate (GenBank accession LT840069), synthesized by FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), was used as a positive control for PCR detection of DNA-α. PCR was performed with Ex Taq DNA polymerase (Takara Bio). The PCR conditions to detect DNA-A were 98 °C for 2 min, followed by 35 cycles of 10 s at 98 °C, 30 s at 45 °C, and 4 min at 72 °C. The PCR conditions for DNA-β were 98 °C for 2 min, followed by 35 cycles of 10 s at 98 °C, 30 s at 45 °C, and 2 min at 72 °C, and conditions for DNA-α were 98 °C for 2 min, followed by 35 cycles of 10 s at 98 °C, 30 s at 50 °C, and 2 min at 72 °C.

All PCR products were separated by 1% agarose gel electrophoresis. Amplicons of the expected size for DNA-A (approximately 2,700 bp) and DNA-β (approximately 1,300 bp) from all AYVV samples were observed (Fig. 3a and b), whereas there were no amplicons of the expected size for DNA-α in any common bean sample (Fig. 3c) or in a tomato sample infected with the AYVV-Ishigaki isolate (Fig. 3c).

Expected DNA fragments were purified using a QIAquick PCR Purification Kit (Qiagen) or a QIAquick gel extraction kit (Qiagen). The nucleotide sequences of extracted DNA fragments were determined using a BigDye Terminator v1.1 Cycle Sequencing Ready Reaction Kit and a Genetic Analyzer DNA Model 3100 (Thermo Fisher Scientific). The nucleotide sequences were collected using Geneious version R10 software (http://www.geneious.com). Primers used for sequencing are listed in Table 1. According to a BLASTn analysis, the partial and complete nucleotide sequences shared 100% and 99.9%, respectively, with the DNA-A sequence of the AYVV isolate previously reported in Ishigaki Island (GenBank accession AB306314). The partial and complete nucleotide sequences were deposited in DDBJ/EMBL/NCBI (GenBank Accessions LC475505 and LC487406).

According to a BLASTn analysis of the complete nucleotide sequence of DNA-β, the nucleotide sequence of the fragments shared 97.9% identity with the complete sequence of DNA-β of the TYLCV isolate reported in Taiwan (GenBank Accession AJ542495). The nucleotide sequence was deposited in DDBJ/EMBL/NCBI (GenBank accession LC48883).

Healthy *P. vulgaris* cv. Bikkuri Jumbo seedlings were used in a transmission test with *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED). *Ageratum conyzoides* plants infected with AYVV were collected from around a common bean field for source of acquisition by whitefly. Single infection of the AYVV was confirmed by PCR as described above (data not shown). Fifty adult whiteflies were given an acquisition access period of 4 days on *A. conyzoides* leaves infected with AYVV. They were then collected and transferred to healthy common bean seedlings (6 viruliferous whiteflies per plant) for an inoculation access period of 2 weeks, after which whiteflies were killed with the insecticide Dinotefuran (Mitsui Chemicals, Tokyo, Japan). Plants were then grown for 2 months in the greenhouse. Healthy plants and nonviruliferous whiteflies were used as controls. The results of this transmission test showed that the virus was transmitted by both *B. tabaci* MEAM1 and MED, and leaf curling and yellowing similar to those observed in the original plants were seen on all test plants (Fig. 1b and Table 2). Additionally, AYVV was detected by PCR in diseased plants but not in asymptomatic healthy plants. We thereby confirmed AYVV transmission from diseased plants to healthy plants via the insect vector *B. tabaci* MEAM1 and MED.

Table 1 Primers used to detect and sequence genomes of ageratum yellow vein virus (AYVV) and tomato yellow leaf curl virus (TYLCV)

Virus	Segment	Primer name	Sequence (5′–3′)	Position[a]	Annealing temperature	Extension time	Agarose concentration	References
AYVV	DNA-A	AYmulti-V	AAGTGGTCCCTACCACGAAC	26–45	53 °C	2 min	2%	–
		AYmulti-C	GAGAGTATGTCTGTTCAACTAAT	232–210				–
		C	AARGAATTCAT-KGGGGCCCCARARRGACTGGC	1790–1760	45 °C	4 min	1%	Briddon and Markham (1994)
		V	KSGGGTCGACGTCATCAATGACGTTRTAC	1808–1836				Briddon and Markham (1994)
		AYVV-AF3	CCAGACTGTACAGAATGTAC	453–472	For sequencing			–
		AYVV-AF4	ATCATCAAGAAGCTGCTAAG	930–949	For sequencing			–
		AYVV-AF5	AATGGCCTCTCGTGGTGCTT	1352–1371	For sequencing			–
		AYVV-AR1	TGGAGACCCATCAGTATTGTG	1950–1970	For sequencing			–
		AYVV-AF1	GATCGTCCATCGATCTGAAAC	2239–2259	For sequencing			–
		AYVV-AF2	AATGCTCTAGCAATCGGTGT	2630–2649	For sequencing			–
	DNA-β	Beta01	GGTACCACTACGCTACGCAGCAGCC	1287–1311	45 °C	2 min	1%	Briddon et al. (2002)
		Beta02	GGTACCTACCCTCCCAGGGGTACAC	1292–1268				Briddon et al. (2002)
		AYVV-betaR2	GGTGTGGGTCCCACCTGGAA	45–26	For sequencing			–
		AYVV-betaR1	CCAAGAGGCTCCAGTAATAG	261–242	For sequencing			–
		AYVV-betaF3	CGAATCTCTATACATGATCC	318–337	For sequencing			–
		AYVV-betaF4	ATTAGTGTATGTATGACTAG	654–673	For sequencing			–
		AYVV-betaF5	CTATCTATTCAGAGAAAATG	786–805	For sequencing			–
		AYVV-betaF1	CTGTTAACAATAATTACCGCCG	985–1006	For sequencing			–
		AYVV-betaF6	AAAGATGTGTCTGTAAGGCGC	1148–1168	For sequencing			–

Table 1 (continued)

Virus	Segment	Primer name	Sequence (5′–3′)	Position[a]	Annealing temperature	Extension time	Agarose concentration	References
		AYVV-betaF2	CTTAGCTAC GCCGGAGCT TA	1311–1330	For sequencing			–
	DNA-α	DNA101	CTGCAGATA ATGTAGCTT ACCAG	713–735	50 °C	2 min	1%	Bull et al. (2003)
		DNA102	CTGCAGATC CTCCACGTG TATAG	718–696				Bull et al. (2003)
TYLCV	DNA-A	TYCON-C	CAACGAACA ATACCAGTA TGCTT	582–560	53 °C	2 min	2%	–
		TYMld-V3	TTCGGTATTAAT TCTTTAATG ATTC	2321–2345				–
		TYIL-V3	TTGGCCTATATA TTAAATAAA CGA	2587–2610				–

[a]Nucleotide position corresponds to the genomes of AYVV isolates (Accessions AB306314 for DNA-A, AB306522 for DNA-β, and KC677736 for DNA-α), and TYLCV isolates (Accession AB363566 for TYCON-C and TYIL-V3 primers and AB116634 for TYMld-V3 primer)

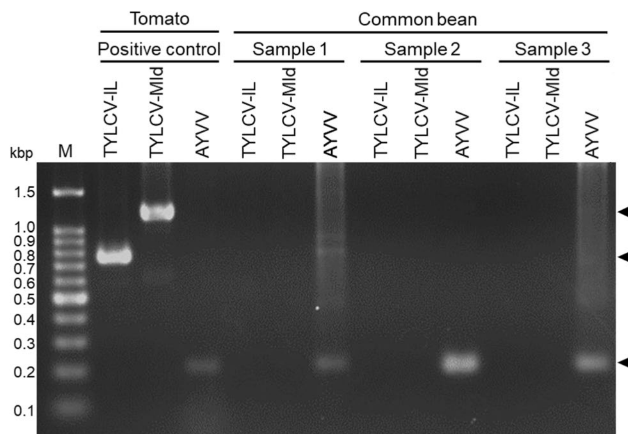

Fig. 2 PCR detection of ageratum yellow vein virus (AYVV) and tomato yellow leaf curl virus (TYLCV) from diseased common beans using specific primers. Tomato plants infected with TYLCV-Israel (IL), TYLCV-Mild (Mld), or AYVV-Ishigaki isolates were used as positive control. Primers are presented in Table 1. PCR products were separated by 2% agarose electrophoresis. Expected amplified products are marked by arrowheads. Lane M, 100 bp DNA ladder (Promega, Madison, WI, USA)

The results of the present study indicate that the causative agent of leaf curling and yellowing disease is AYVV. The first report of AYVV was from *A. conyzoides* in Singapore (Swanson et al. 1993). Since then, the virus has been reported in several dicotyledonous plants (Liu et al. 2008; Tsai et al. 2011). In Japan, AYVV was reported in tomato plants in Okinawa and Tokyo Prefectures (Andou et al. 2010; Shahid et al. 2014). The present study is the first observation of AYVV in a common bean plant. Andou et al. (2010) reported that *A. conyzoides* was an important reservoir of AYVV. Notably, we found *A. conyzoides* infected with AYVV around the greenhouse in which the original infected bean plants were growing; thus, AYVV likely transmitted by whiteflies from *A. conyzoides* to the bean plants. Therefore, management of weeds and whitefly are likely to be the most important control measures of this disease. Because AYVV causes serious damage to tomato and common bean plants, it is essential to carefully monitor the spread of AYVV in other areas, including the

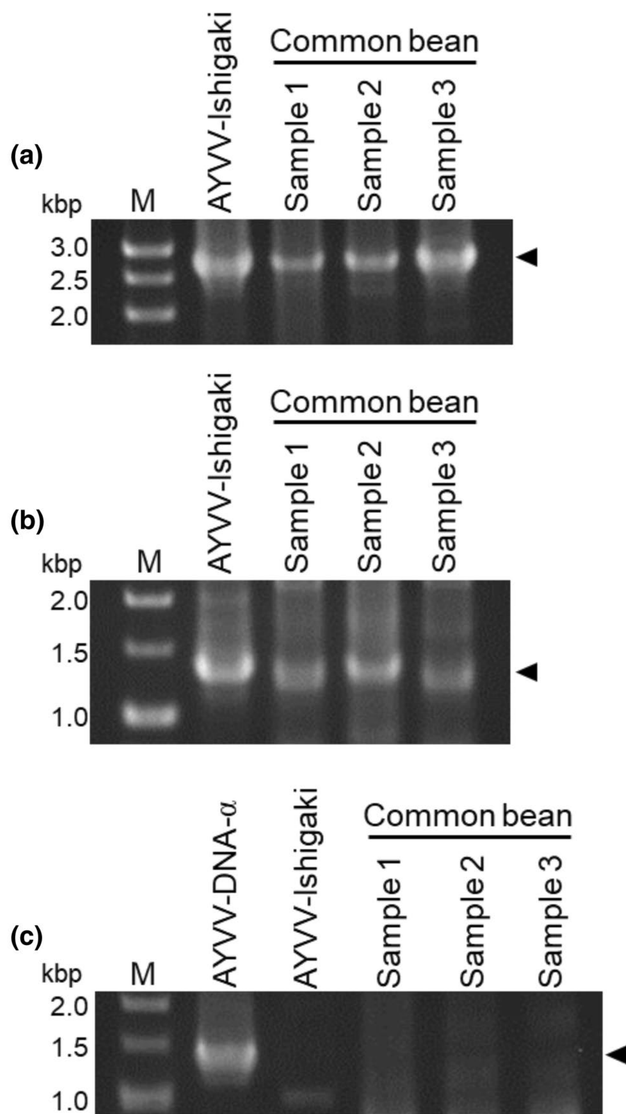

Fig. 3 PCR detection of DNA-A (**a**), DNA-β (**b**), and DNA-α (**c**) of ageratum yellow vein virus (AYVV) from diseased common beans. Tomato plants infected with AYVV-Ishigaki isolate were used as a positive control. In PCR detection of DNA-α of AYVV, synthesized DNA-α of AYVV-Pakistan isolate (GenBank Accession LT840069) was also used as a positive control. Primer sets are in Table 1. PCR products were separated by 1% agarose gel electrophoresis. Expected amplified products are marked by arrowheads. Lane M, 1 kbp DNA ladder (Promega)

Table 2 Test for transmission of ageratum yellow vein virus by genetic groups of *Bemisia tabaci*

Genetic group	Viral status of whiteflies[a]	No. diseased plants/No. inoculated plants[b]
MEAM1	+	4/4
	–	0/4
MED	+	4/4
	–	–[c]

[a]+, Viruliferous; –, nonviruliferous

[b]AYVV was detected in all diseased plants by PCR

[c]Experiment was not done

main island of Okinawa. To the best of our knowledge, this is the first report of leaf curling and yellowing caused by AYVV in *P. vulgaris* in Japan. We therefore propose adding yellow leaf curl disease as a new disease of common bean.

Acknowledgements We thank Ms. Kazue Sato and Ms. Mariko Nagata for maintaining plants, insects and other materials. We are deeply grateful to Professor Souta Koeda for providing DNA of AYVV isolate. We also thank Mr. Takaaki Ikeshiro for helpful comments and technical assistance about the transmission of the virus by insect.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights statement This article does not contain any studies with human participants or animals performed by any of the authors.

References

Andou T, Yamaguchi A, Kawano S, Kawabe K, Ueda S, Onuki M (2010) *Ageratum yellow vein virus* isolated from tomato plants with leaf curl on Ishigaki Islands, Okinawa, Japan. J Gen Plant Pathol 76:287–291

Briddon RW, Markham PG (1994) Universal primers for the PCR amplification of dicot-infecting geminiviruses. Mol Biotech 1:202–205

Briddon RW, Bull SE, Mansoor S, Amin I, Markham PG (2002) Universal primers for PCR-mediated amplification of DNA β. Mol Biotech 20:315–318

Bull SE, Briddon RW, Markham PG (2003) Universal primers for the PCR-mediated amplification of DNA 1: a satellite-like molecule associated with begomovirus-DNA β complexes. Mol Biotech 23:83–86

Liu Z, Yang CX, Jia SP, Zhang PC, Xie LY, Xie LH, Lin QY, Wu ZJ (2008) First report of *Ageratum yellow vein virus* causing tobacco leaf curl disease in Fujian Province, China. Plant Dis 92:177

Shahid MS, Ikegami M, Waheed A, Briddon RW, Natsuaki KT (2014) Association of an alphasatellite with *Tomato yellow leaf curl virus* and *Ageratum yellow vein virus* in Japan is suggestive of a recent introduction. Viruses 6:189–200

Swanson MM, Harrison BD, Wong SM (1993) A geminivirus causing vein yellowing of *Ageratum conyzoides* in Singapore. Plant Pathol 42:137–139

Tsai WS, Shih SL, Venkatesan SG, Aquino MU, Green SK, Kenyon L, Jan FJ (2011) Distribution and genetic diversity of begomoviruses infecting tomato and pepper plants in the Philippines. Ann Appl Biol 158:275–287

Ueda S, Onuki M, Kijima K, Futagami K, Kinjo K, Murayama Y, Taniguchi M, Kawano S (2009) Introduction and molecular characterization of *Tomato yellow leaf curl virus* in Okinawa, Japan. JARQ 43:19–24

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.