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SHORT NOTES

Viruses infecting periwinkle (*Catharanthus roseus* L.) in Western Saudi Arabia

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Summary. Periwinkle (*Catharanthus roseus* L.) is an ornamental plant widely grown in the Mecca region in the Kingdom of Saudi Arabia (KSA). Different symptoms similar to those induced by viruses (mosaic, vein clearing, mottling, yellowing, flower variegation) prompted surveys in eight different regions of KSA to assess the presence of periwinkle-infecting viruses, i.e. *Tomato spotted wilt virus* (TSWV), *Cucumber mosaic virus* (CMV), *Lettuce mosaic virus* (LMV), *Catharanthus mosaic virus* (CatMV) and *Catharanthus yellow mosaic virus* (CYMV). Two hundred and forty leaf samples were collected from naturally infected nurseries, gardens and landscape plantings, and tested by DAS-ELISA, RT-PCR and PCR assays. Results showed that all five viruses were present in the surveyed areas, with incidence of infection reaching 58%. TSWV was the most widespread, found in 44% of samples, followed by CYMV (35%), whereas CatMV (15%), CMV (11%) and LMV (6%) were present to lesser extents. The virus incidence ranged between 43% in Rabigh to 77% in Taif. This is the first report of CYMV and CatMV in KSA, and of TSWV, LMV and CMV in periwinkle in western Saudi Arabia. The high incidence of viruses and of multiple infections in periwinkle plants endangers this crop in KSA, so a sanitation programme for eliminating virus infections is desirable.

Key words: Mecca region, virus detection, ELISA PCR.

Introduction

Catharanthus roseus (*Apocynaceae* family), also known as the common periwinkle or Madagascar periwinkle, is a perennial evergreen subshrub cultivated worldwide. This plant has historically been used in popular medicine to treat a wide assortment of human diseases, including diabetes and high blood pressure, as it contains more than 150 useful alkaloids (Favali *et al*., 2004; Jaleel *et al*., 2006; Kulkarni *et al*., 2016). Periwinkle is also important for decoration of gardens and fences.

Several diseases caused by bacteria, fungi, nematodes, phytoplasmas and viruses are reported on periwinkle from different parts of the world. Among many viruses reported to infect periwinkle in nature (including *Arabis mosaic virus*, *Tobacco ringspot virus*, *Tomato chlorotic spot virus*, *Zantedeschia mild mosaic virus*, *Potato yellow vein virus*, *Potato yellow dwarf virus*), *Tomato spotted wilt virus* (TSWV), *Cucumber mosaic virus* (CMV), *Catharanthus mosaic virus* (CatMV), *Catharanthus yellow mosaic virus* (CYMV) and *Lettuce mosaic virus* (LMV) remain among the most important as being associated with serious periwinkle diseases (Falk *et al*., 1981; Chatzivassiliou *et al*., 2000; Salazar *et al*., 2000; Huang and Chang, 2005; Singh *et al*., 2005; Maciel *et al*., 2011; Fisher, 2013; Warfield *et al*., 2015).

Several reports of periwinkle plants exhibiting mosaic, vein clearing and mottling, leaf curling and yellowing, all symptoms induced by viruses,

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prompted a survey in nurseries, gardens, orchards and landscape plantings in eight different regions (Taif, Makkah, Jeddah, Bahra, Khulais, Aljummum, Lith and Rabigh) of the Kingdom of Saudi Arabia (KSA), to assess the presence and incidence of TSWV, CMV, CatMV, CYMV and LMV. This paper reports results of that survey.

Materials and methods

Plant source material

The survey was conducted in May 2016, and leaves from 240 plants were collected from four breeding series of periwinkle varieties (Pacifica, Titan, Viper and Sunstorm). Of these samples, 166 were exhibiting different types of symptoms (Figure 1).

Detection of periwinkle viruses

All samples were assayed serologically by Double Antibody Sandwich Enzyme-Linked Immuno-Sorbent Assay (DAS-ELISA; Clark and Adams, 1977) for the presence of TSWV, CMV, LMV following the manufacturer's instructions (Bioreba AG). CatMV and CYMV were assessed uniquely using molecular assays (respectively, RT-PCR and PCR), since no commercial antibodies are available. In addition, 5% of samples that were DAS-ELISA-positive for TSWV,

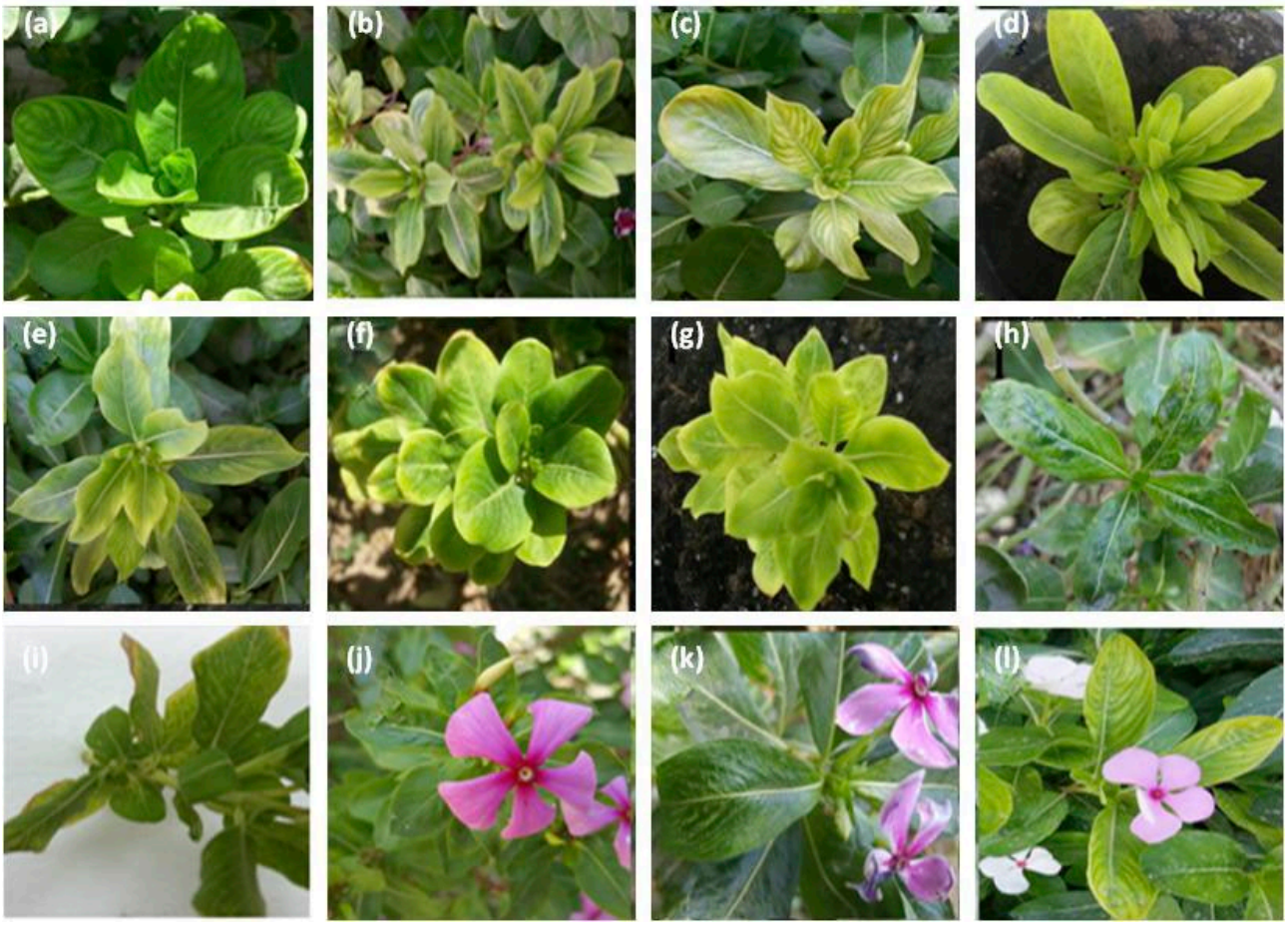

Figure 1. Periwinkle plants exhibiting different types of symptoms observed in inspected nurseries, gardens, orchards and landscape plantings in different provinces of the Kingdom of Saudi Arabia. (a) healthy periwinkle plant; (b) leaf distortion; (c) chlorosis; (d) yellowing; (e) deformation of apical leaves; (f) fleck (g) mottling; (h) mosaic; (i) rosette and stunting; (j) blotching and flower distortion; (k) vein clearing and flower variegation; (l) vein necrosis and mottling.

Table 1. List of virus-specific primers used in PCR\RT-PCR. Nc: Nucleocapsid protein; CP: capsid protein; RdRp: RNA-dependent RNA polymerase.

Virus	Gene target	Primer sequences (5′ to 3′)	Amplicon size (bp)	Reference
TSWV	Nc	ATGTCTAAGGTTAAGCTC TTAAGCAAGTTCTGTGAG	800	Holguín and Rueda (2007)
CMV	RdRp	TAACCTCCCAGTTCTCACCGT CCATCACCTTAGCTTCCATGT	513	Grieco *et al*. (2000)
LMV	CP-3′UTR	ACAAGAAGAAACCGTATATGCC GCCAACACACGCCTTTAGTG	297	Revers *et al*. (1997)
CatMV	CP	TGAACGGACTGATGGTATGG CCTCACTCATCTGGAACTTC	650	Conceicao *et al*. (2011)
CYMV	CP	TGTCGAAGCGACCAGCAGATAT TTAATTCGATACTGAATCATAAAA	771	Ilyas *et al*. (2013)

CMV and LMV were retested by RT-PCR for confirmation. For the molecular tests, total RNAs were extracted from leaf veins using 100 mg of tissues that were macerated in 1 mL of grinding buffer (4.0M guanidine thiocyanate, 0.2M NaOAc pH 5.2, 25mM EDTA, 1.0M KOAc and 2.5% (w/v) PVP-40), and purified using silica particles as described by Foissac *et al*. (2001). Total DNA in samples was extracted using the cetyl trimethyl ammonium bromide (CTAB) method (Sambrook and Russell, 2001). Total RNAs were reverse-transcribed with 0.5 μg of random hexamer primers (Boehringer) in the presence of 200 units of *Moloney murine leukaemia virus* (M-MLV) reverse-transcriptase enzyme (Bethesda Research Laboratories) for 1 h at 39°C. The cDNA synthesized was further used in RT-PCR to detect TSWV, CMV, LMV and CatMV. CYMV was checked by PCR applied to CTAB-extracted samples. Five virus-specific primers pairs were used in PCR and RT-PCR, with the nucleotide sequences outlined in Table 1, together with references that report their PCR use conditions. Amplification products were analyzed by electrophoresis on 1.2% agarose gel prepared in 1 × TAE buffer, stained with ethidium bromide and examined on UV transilluminator. PCR amplicons generated from the amplification of CatMV and CYMV were ligated into the pGEM-T Easy vector according to the manufacturer's instructions (Promega), and were used to transform *Escherichia coli* DH5α-competent cells. Selected clones were subjected to automated sequencing (Primm) and BLASTn search at the NCBI website (Altschul *et al*., 1990).

Results and discussion

ELISA and PCR results showed that all the five assayed viruses were present in the surveyed areas, with different incidence of infections. From the 240 samples tested, 140 (58%) were infected by at least one virus. Among the investigated viruses, TSWV was the most widespread with incidence of 44% (Table 2), and TSWV incidence was particularly high in Taif (67%) and Makkah (57%) regions, where the periwinkle variety "Pacifica" showed to be almost completely infected (90% in Taif and and 100% in Makkah). This variety was particularly compromised since it harboured all the viruses tested, and was followed by cvs. "Viper" and "Sunstorm" (Table 2). CYMV ranked second (35% overall incidence), and was present in all regions except Rabigh. RT-PCR results showed that CatMV was present in 15% of the samples, and was absent in the Rabigh and Lith regions. CMV (11% of samples) and LMV (6%) were the least prevalent viruses.

The RT-PCR analyses carried out on 5% of ELISA-positive samples for TSWV, CMV and LMV confirmed the positive reactions (Figure 2). In the cases of CatMV and CYMV, for which no serological kits are commercially available, sequences of PCR amplicons from two randomly selected positive samples showed that they were identical to sequences deposited in the Genbank for CatMV (acc.nos DQ365928 for CatMV and HE580234 for CYMV).

The high incidence of virus infections (58%) in periwinkle plants from KSA observed in this study

Table 2. Incidence of CYMV, CatMV, TSWV, CMV and LMV in tested samples, according to DAS-ELISA, PCR and RT-PCR assays conducted samples from 240 periwinkle plants of different breeding series, from eight Kingdom of Saudi Arabia provinces.

Province	Breeding Series	Tested plants No.	Infected plants No.		CYMV		CatMV		CMV		LMV		TSWV	
			N	%	N	%	N	%	N	%	N	%	N	%
Jeddah	Pacifica	7	6	85.71	5	71.43	3	42.86	6	85.71	1	14.29	1	14.29
	Titan	9	4	44.44	2	22.22	1	11.11	2	22.22	0	00.00	0	00.00
	Sunstorm	9	5	55.56	3	33.33	2	22.22	4	44.44	1	11.11	1	11.11
	Viper	5	4	80.00	4	80.00	1	25.00	3	60.00	0	00.00	1	20.00
	Total Samples	30	19	63.33	14	46.67	7	23.33	15	50.00	2	06.67	3	10.00
Makkah	Pacifica	7	7	100	6	85.71	3	42.86	7	100	2	28.57	1	14.29
	Titan	9	5	55.56	3	33.33	1	11.11	3	33.33	1	11.11	1	11.11
	Sunstorm	10	6	60.00	4	40.00	3	30.00	4	40.00	2	20.00	0	00.00
	Viper	4	3	75.00	2	50.00	1	25.00	3	75.00	1	25.00	1	25.00
	Total Samples	30	21	70.00	15	50.00	8	26.67	17	56.67	6	20.00	3	10.00
Taif	Pacifica	10	9	90.00	7	70.00	3	30.00	9	90.00	3	30.00	1	10.00
	Titan	10	5	50.00	3	30.00	1	10.00	4	40.00	1	10.00	0	00.00
	Sunstorm	6	5	83.33	5	85.33	3	50.00	4	66.67	3	50.00	2	33.33
	Viper	4	4	100	4	100	1	25.00	3	75.00	1	25.00	2	50.00
	Total Samples	30	23	76.67	19	63.33	8	26.67	20	66.67	8	26.67	5	16.67
Aljumum	Pacifica	8	5	62.50	3	37.50	1	12.50	4	50.00	1	12.50	0	00.00
	Titan	5	2	40.00	1	20.00	1	20.00	1	20.00	1	20.00	0	00.00
	Sunstorm	10	4	40.00	3	30.00	1	10.00	4	40.00	0	00.00	0	00.00
	Viper	7	4	57.14	3	42.86	1	14.29	4	57.14	0	00.00	0	00.00
	Total Samples	30	15	50.00	10	33.33	4	13.33	13	43.33	2	06.66	0	00.00
Rabigh	Pacifica	4	2	50.00	0	00.00	0	00.00	2	50.00	1	25.00	0	00.00
	Titan	12	4	33.33	0	00.00	0	00.00	1	25.00	0	00.00	0	00.00
	Sunstorm	10	4	40.00	0	00.00	0	00.00	2	20.00	1	10.00	0	00.00
	Viper	4	3	75.00	0	00.00	0	00.00	2	20.00	1	25.00	0	00.00
	Total Samples	30	13	43.00	0	00.00	0	00.00	7	23.33	3	10.00	0	00.00
Bahra	Pacifica	12	9	75.00	6	50.00	3	25.00	8	66.67	3	25.00	1	08.33
	Titan	10	4	40.00	3	30.00	2	20.00	4	40.00	1	10.00	1	10.00
	Sunstorm	6	4	66.67	4	66.67	2	33.33	4	66.67	2	33.33	1	16.67
	Viper	2	1	50.00	0	00.00	1	50.00	0	00.00	0	00.00	0	00.00
	Total Samples	30	18	60.00	13	43.33	8	26.67	16	53.33	6	20.00	3	10.00

(Continued)

Table 2. (Continued).

Province	Breeding Series	Tested plants No.	Infected plants No.		CYMV		CatMV		CMV		LMV		TSWV	
			N	%	N	%	N	%	N	%	N	%	N	%
Khulais	Pacifica	10	8	80.00	2	20.00	1	10.00	5	50.00	0	00.00	0	00.00
	Titan	5	2	40.00	2	40.00	0	00.00	1	20.00	0	00.00	0	00.00
	Sunstorm	10	4	40.00	0	00.00	0	00.00	3	30.00	0	00.00	0	00.00
	Viper	5	2	40.00	0	00.00	0	00.00	2	40.00	0	00.00	0	00.00
	Total Samples	30	16	53.33	4	13.33	1	03.33	11	36.67	0	00.00	0	00.00
Lith	Pacifica	10	7	70.00	5	50.00	0	00.00	1	10.00	0	00.00	0	00.00
	Titan	3	1	33.33	0	00.00	0	00.00	1	33.33	0	00.00	0	00.00
	Sunstorm	10	4	36.36	3	27.27	0	00.00	2	18.18	0	00.00	0	00.00
	Viper	7	3	42.86	1	14.29	0	00.00	2	28.57	0	00.00	0	00.00
	Total Samples	30	15	50.00	9	30.00	0	00.00	6	20.00	0	00.00	0	00.00
	Total	240	140	58.33	84	35.00	36	15.00	105	43.75	27	11.25	14	5.83
Mean infection rate			58.33		35.00		15.00		43.75		11.25		5.83	

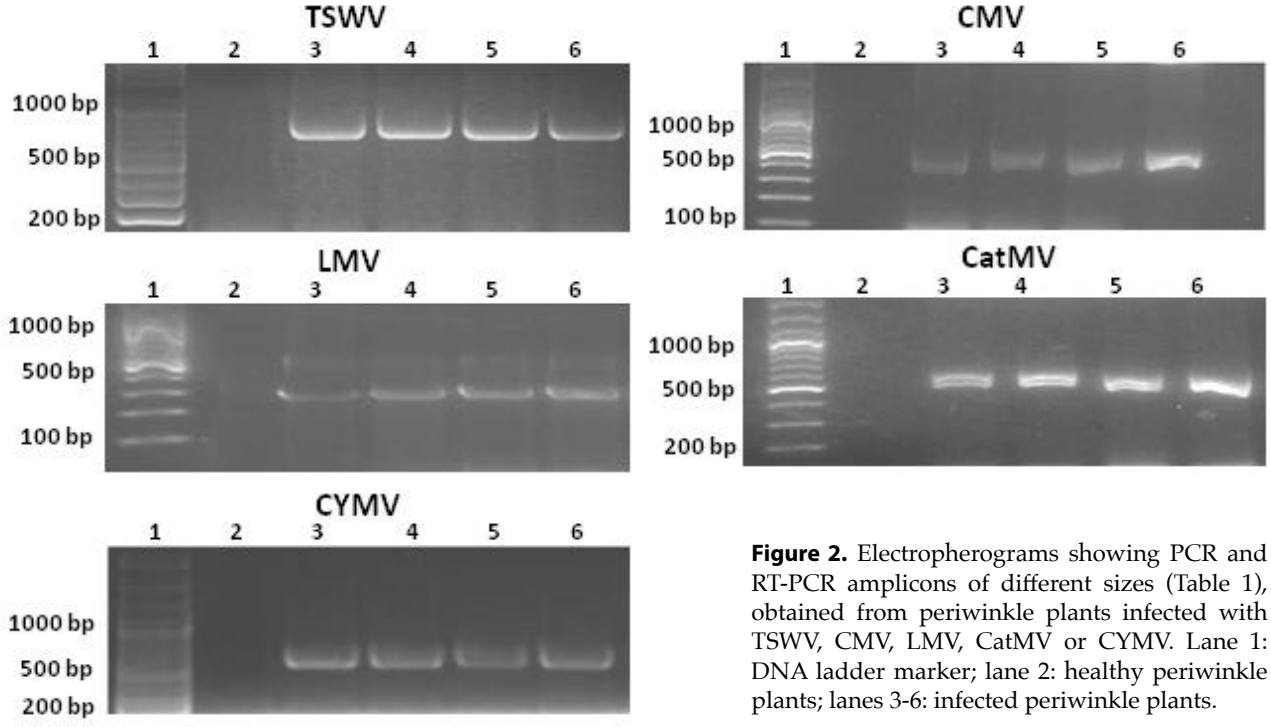

Figure 2. Electropherograms showing PCR and RT-PCR amplicons of different sizes (Table 1), obtained from periwinkle plants infected with TSWV, CMV, LMV, CatMV or CYMV. Lane 1: DNA ladder marker; lane 2: healthy periwinkle plants; lanes 3-6: infected periwinkle plants.

were expected, given the prevalence of typical symptoms observed in nurseries and landscape plantings. Also expected was the high incidence of TSWV in periwinkle plants (44%), given the recent report of this virus in infected lettuces in KSA (Al-Saleh *et al*., 2014), and its large host range of more than 550 species in 62 different monocot and dicot plant families, including ornamental plants, vegetables and many weed species (Sether *et al*., 1992). Such high incidence of virus infections, in particular of TSWV, unveiled the disease etiology in most of the diseased plants seen during our survey in different locations of KSA. However, the presence of mixed infections in many cases rendered it difficult to the interpret the respective symptoms. It was not possible to determine the cause of some virus-like symptoms observed in 26 diseased periwinkle plants, which were negative for all the viruses here investigated. Whether these symptoms were due to infectious diseases of biotic nature, i.e. other viruses, or bacteria, phytoplasmas or to other abiotic factors, is yet to be determined.

The outcome of this preliminary study extends knowledge of the presence and spread of periwinkle viruses in Saudi Arabia, for which no previous information was available. This study reports for the first time the presence of CYMV and CatMV in the KSA, especially in the Mecca regions, and the prevalence and distribution of TSWV, CMV and LMV in this country. The knowledge on viral diseases of periwinkle in the Mecca region of Saudi Arabia should prompt a suitable sanitary sanitation programme for the production of healthy plant material.

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