Iran Agricultural Research (2023) 42(1) 47-54

Short Communication

Shiraz University

First report of the incidence of potato virus Y in some ornamental plants in Iran

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DOI: 10.22099/IAR.2023.48019.1550

ARTICLE INFO
***Article history*:**
Received **06 August 2023**
Accepted **07 November 2023**
Available online **11 November 2023**

***Keywords*:**
Degenerate primers
Green space
Potyvirus
Ornamental plants
Shiraz

ABSTRACT- During the surveys conducted from green space in Shiraz City, Iran, in the summer of 2022, leaf chlorosis and mosaic symptoms were observed on the leaves of black-eyed-susans (*Rudbeckia hirta*), a *Dahlia* sp., and Mexican Petunia (*Ruellia brittoniana*) plants. Total genomic RNA was separately extracted from 10 symptomatic and one symptomless (negative control) leaf samples and subjected to Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using a potyvirus degenerate primer pair (NIb3R, NIb2F). RT-PCR resulted in the amplification of an approximately 350 bp DNA fragment in all symptomatic samples, while no such fragment was amplified from the symptomless plant. The amplified DNA fragment was subjected to Sanger sequencing, and its size was determined to be exactly 350 bp, and confirmed that it belongs to the Nib gene of potato virus Y (PVY). The nucleotide (nt) sequence of the amplicons was compared with the nt sequence of the same region of some other PVY isolates that were available in the GenBank. The sequence analysis revealed that the *R. brittoniana* isolate exhibited the highest (98.8%) similarity to a PVY isolate from the USA with the Acc. No. of KY_848029.1. Similarly, the *R. hirta* and the *Dahlia* isolates showed the highest (98.3%, 98.4 %, respectively) similarity to a PVY isolate from Kazakhstan with the Acc. No. of ON_583980.1.

Perennial ornamental plants are widely used in landscape design, but unfortunately, these plants are susceptible to various species of plant viruses. These viruses pose significant challenges to the production, decorative value, and quality of ornamental crops, leading to substantial losses and making it difficult to control them Additionally, the introduction of ornamental plants to new areas can give rise to new viral diseases. It has been reported that infection of ornamental plants with viruses can result in various symptoms that lead to a decline in the quality of propagating materials and substantial financial losses to these plants (Valverde et al., 2012). Among the ornamental plants vulnerable to viral infection, black-eyed-susans (*Rudbeckia hirta*), species of dahlia (*Dahlia* spp.), and Mexican Petunia (*Ruellia brittoniana*) are extensively cultivated for their aesthetic appeal. Recently, potato virus Y (PVY), the type member of the genus *Potyvirus* (Quenouille et al, 2013; https://ictv.global/report/chapter/potyviridae/potyviridae/potyvirus) has been identified as an infecting agent of these plants (Hussain et al., 2016), which can cause significant concern and have a substantial impact in horticultural environments (Alexandre *et al*., 2021).

Among the various plant virus groups, the Potyvirus genus is one of the largest groups, encompassing 193 definitive species and 28 possible members (related-unclassified viruses) with the ability to infect a wide variety of ornamental plant species (https://ictv.global/report/chapter/potyviridae/potyviridae/potyvirus). Some of these viruses are responsible for causing significant losses in agricultural, pasture, horticultural, and ornamental crops (Sharma et al., 2013). This study was focused on the isolation, detection, sequencing, and identification of PVY, isolates from some ornamental plants. The findings of the current study shed light on the impact of this potyvirus on some ornamental crops and certainly contribute to understanding the role of this virus in the ornamental plants sector in horticulture. Previously, various potyviruses have been reported from ornamental plants. The host plant species and infecting potyvirus(es) are listed in Table 1.

Table 1. List of potyviruses reported from ornamental plants around the world

Ornamental host plant	Potyvirus name	Reference(s)
Alstroemeria spp.	Alstroemeria mosaic virus	(Pearson et al., 2009)
Amaryllis spp.	Nerine yellow stripe virus	(Pearson et al., 2009)
Anthurium spp.	Dasheen mosaic virus	(Alexandre *et al*., 2023)
Brugmansia spp.	Brugmansia suaveolens mottle virus; Columbian datura virus	(Jordan et al., 2011; Rott et al., 2009; Salamon, & Palkovics, 2005; Steele and Thomas, 2009; Verma et al., 2014)
Caladium bicolor	Dasheen mosaic virus	(Alexandre *et al*., 2023)
Canna spp.	Bean yellow mosaic virus; Canna yellow streak virus; sugarcane mosaic potyvirus	(Alexandre *et al*., 2023; Kumari et al., 2022; Mitrofanova et al., 2018; Salehzadeh et al., 2023)
Catharanthus roseus	Catharanthus mosaic virus;	(Maciel et al., 2015)
Chrysanthemum	Turnip mosaic virus, zucchini yellow mosaic virus, Chrysanthemum spot virus, potato virus Y; soybean mosaic virus	(Liu et al., 2014; Mitrofanova et al., 2018)
Costus spiralis	Costus stripe mosaic virus	(Alexandre *et al*., 2023)
Cotyledon orbiculata	Cotyledon virus Y (Related, unclassified potyvirus**)**	(Duarte et al., 2014)
Dasheen spp.	Dasheen mosaic virus	(Chen et al., 2001)
Datura inoxia	Colombian datura virus	(Verma et al., 2014)
Dieffenbachia	Dasheen mosaic virus	(pandit et al., 2001)
Freesia spp.	Bean yellow mosaic virus*; Freesia* mosaic virus (syn. *Spiranthes* mosaic virus 2)	(Pearson et al., 2009
Eucharis grandiflora	Hippeastrum mosaic virus	(Alexandre *et al*., 2023)
Euphorbia spp.	Euphorbia ringspot virus	(Jordan et al., 2011; Marys & Romano, 2011)
Helianthus annuus	Bidens mosaic virus; sunflower chlorotic mottle virus	(Alexandre *et al*., 2023) (Bello et al., 2023)
Gloriosa superba	Gloriosa stripe mosaic virus	(Mollov et al., 2017)
Hippeastrum spp.	bean yellow mosaic virus; Hippeastrum mosaic virus	(Alexandre et al., 2023)
Hippeastrum hybridum	Amazon lily mosaic virus; Hippeastrum mosaic virus	(Raj et al., 2009)
Hymenocallis	Nerine yellow stripe virus	Pham et al., 2011
Hyacinth sp.	Hyacinth mosaic virus	(Alexandre et al., 2017)
Impatiens walleriana	Impatiens flower break virus	(Cho et al., 2017; Jordan et al., 2011)
Iris spp.	Bean yellow mosaic virus ; Iris mild mosaic virus; Iris severe mosaic virus; Ornithogalum mosaic virus	(Asjes 1979; Kulshrestha et al., 2006 *;* Pearson et al., 2009)
Kalanchoe blossfeldiana	Kalanchoe mosaic virus	(Duarte et al., 2014)
Lachenalia spp.	Ornithogalum virus 3; Veltheimia mosaic virus (Related, unclassified potyvirus)	(Pearson et al., 2009)
Lilium spp.	Freesia mosaic virus; lily mottle virus	(Pearson et al., 2009)
Mandevilla sp.	Catharanthus mosaic virus	(Alexandre *et al*., 2023)
Masdevallias sp.	Bean yellow mosaic virus	(Koenig, 1984)
Narcissus spp.	Hippeastrum mosaic virus; Narcissus degeneration virus; Narcissus late season yellows virus; Narcissus yellow stripe virus;	(Ágoston et al., 2020*;* Chen et al 2006; Ward et al., 2009)
Narcissus tazetta	Narcissus yellow stripe virus	Raj et al., 2019
Nerine spp.	Nerine yellow stripe virus; Vallota mosaic virus;	(Pearson et al., 2009*;*Pham et al., 2011)
Omphalodes sp.	Omphalodes virus Y	(Jordan et al., 2011)
Ornithogalum thyrosoides	Ornithogalum mosaic virus	(Pearson et al., 2009)
Osteospermum	Lettuce mosaic virus	(Jordan et al., 2011)
Philodendron	Dasheen mosaic virus	(Pandit et al., 2001)
Polianthes tuberosa	tuberose mild mosaic virus	(Chen et al., 1998)
Schizostylis	Bean yellow mosaic virus	(Jordan et al., 2011)
Spiranthes	Dasheen mosaic virus, Spiranthes mosaic virus 2, Spiranthes mosaic virus 3	(Jordan et al., 2011)
Stenomesson	Nerine yellow stripe virus	(Jordan et al., 2011)
Tradescanthia spathacea	Costus stripe mosaic virus	(Alexandre *et al*., 2023; Favara et al., 2021)
Tricyrtis formosana	Tricyrtis virus Y in a mixed infection with lily virus X (potxvirus)	(Jordan et al., 2011)

Table 1. Continued

Ornamental host plant	Potyvirus name	Reference(s)
Vallota speciosa (*Cyrtanthus elatus)*	Nerine yellow stripe virus	(Pham et al., 2011)
Verbena canadensis	Verbena virus Y in a mixed infection with broad bean wilt virus-1 and Coleus vein necrosis virus	(Kraus et al., 2010)
Welwitschia mirabilis	Catharanthus mosaic virus	(Koh et al., 2015)
Zantedeschia aethiopica	Dasheen mosaic virus	(Chen et al., 2001)
Zinnia elegans	Bidens mosaic virus	(Alexandre *et al*., 2023)

These instances highlight the diverse range of ornamental plants susceptible to potyvirus infections, emphasizing the need for effective disease management strategies within the horticultural industry.

In January 2022, a survey was conducted in the green space of Shiraz City to examine the viral diseases of three ornamental plant species including black-eyed-susans (*R. hirta*, Family *Asteraceae*), Dahlia sp. (Family *Cannaceae*), and Mexican Petunia (*R. brittoniana*, Family *Solanaceae*). Symptoms of mosaic and chlorosis were observed on the leaves of these plants (Fig. 1A-C).

To investigate the presence of potential viral infections, a total of 10 symptomatic leaf samples along with one asymptomatic sample were collected for analysis, Total RNA was extracted from these samples using Trizol reagent from Denazist, Iran. The extracted RNA samples were subjected to Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using a potyvirus degenerate primer pair including NIb2F (5'-GTITGYGTIGAYGAYTTYAAYAA-3'), and NIb3R (5'-TCIACIACIGTIGAIGGYTGNCC-3') (Gibbs and Mackenzie 1997) which target an approximately 350 nucleotide (nt) fragment in the Nib gene of the genome of potyviruses. PCR was carried out in a 25 µl reaction mixture containing 12 µl PCR mastermix (Denazist, Iran), 9 µl H_2O, 2 µl of each primer (10 µM), and 2 µl cDNA. The mixture was initially denatured at 94°C for 5 minutes, followed by amplification through 35 cycles, each cycle consisting of denaturation at 94°C for 60 seconds, annealing at 54°C for 60 seconds, and extension at 72°C for 60 seconds, with a final cycle at 72°C for 10 minutes. Subsequently, the amplification products were resolved on 1% (w/v) Tris-acetate agarose gels and stained with ethidium bromide. The RT-PCR analysis yielded a DNA fragment of the expected size (~350 bp) in all of the ten examined symptomatic samples, while no RT-PCR product was obtained from the asymptomatic leaf sample (Fig. 1D). Subsequently, the RT-PCR products from two samples of each tested ornamental plant were purified and directly sequenced by Sinohe Inc., Iran. The obtained DNA sequences were analyzed using the nucleotide Blast Program (NCBI) and then aligned and compared with some other corresponding sequences available in the GenBank using MEGA version V 8.0.

The sequence analysis using the nucleotide Blast search Program (NCBI), confirmed the presence of an RNA fragment belonging to PVY in *R. hirta*, *Dahlia* sp., and *R. brittoniana* plants tested in this study. Nucleotide sequences comparison with other corresponding sequences available in the GenBank databases (Table 1), using MEGA version V 8.0., showed that *R.brittoniana* isolate shared the highest nt identity (98.8 %) with the WI120092 isolate of PVY (Table 1, Acc. No. KY848029) reported from the Wisconsin State in USA which belongs to the PVY^O strain (Green et al., 2017) indicating that the isolate of PVY from *R. brittoniana* is most likely an O strain of PVY. The isolate obtained from *R. hirta* and the *Dahlia* isolate exhibited the highest similarity (98.3 % and 98.4 %, respectively) to the ALYU-76 isolate of PVY from Kazakhstan (Acc. No. ON583980.1, Table 1).

A maximum likelihood phylogenetic tree was generated using a 350 nucleotide segment of the PVY-NIb gene of the three Iranian isolates, characterized in the current study, and 13 PVY sequences, all isolated from potato (*Solanum. Tuberosum*), (retrieved from GenBank; Table 1) using MEGA8.0 Software with 100 bootstrap replicates revealed the presence of two major clusters (Groups 1 and 2 Fig. 2.) Cluster 1 encompasses the PVY isolate from *R. brittoniana* (characterized in this study), along with 23 PVY isolates, primarily originating from the American continent (each with more than 98 % nt identity at the nucleotide level, Table 2), as depicted in Fig. 2 under Group 1. The two other PVY isolates characterized in this study (PVY isolates from *R. hirta* and *Dahlia* sp.) along with four Asian PVY isolates (each with more than 98 % nt identity at the nucleotide level, Table 1), constitute the second cluster (Fig. 2, Group 2).

To identify potential co-infections of PVY with other viruses in the PVY-infected samples, RT-PCR was conducted using four sets of different primer pairs including a primer pair targeting a portion of the coat protein (CP) of cucumber mosaic virus (CMV), (CMV CP-F, CMV CP-R, Rizos et al., 1992), a degenerate primer pair for universal detection of begomoviruses ($primerB^C$, Deng et al., 1994 / $primer181^V$, Rojas et al., 1993), and a degenerate primer pair (TobamodF/TobamodR, Li et al., 2018) to detect viruses of the genus *Tobamovirus.* However, no PCR products were amplified using these primer pairs, implying the absence of mixed infection of PVY with other viruses in the examined plants.

The economic importance of ornamental plant production and the susceptibility of various plant hosts to potyviruses in Iran highlights the significance of identifying and understanding the genetic diversity of viruses associated with ornamental plants. This knowledge is crucial for accurate detection, implementation of improved control management against detected viruses, and prevention of further infections.

Notably, to our knowledge and a review of past research shows that, except the report of strain N-Wilga of PVY infecting *Chrysanthemum* in China (Liu et al., 2014), no ornamental plant has been reported as a host for approximately 4670 confirmed nt sequences of PVY registered in the GenBank and this study represents the

first report of PVY occurrence in ornamental plants; *R. hirta*, *Dahlia* sp., and *R.brittoniana*. The findings of this study provide important insights into the genetic composition and potential origin of the PVY-infected ornamental plants, certainly contributing to the understanding of plant viral infections and their impact on horticulture.

STATEMENTS and DECLERATIONS

Competing Interests

Authors declare they have no financial interests.

Author Contributions

All authors contributed to the study conception and design, Material preparation, data collection and nucleotide sequence analysis were performed by Alireza Afsharifar and Mehrdad Salehzadeh. The first draft of the manuscript was written by Alireza Afsharifar and all authors commented on previous versions of the manuscript. All authors read and approved the final file of the manuscript.

ACKNOWLEDGMENTS

The authors wish to express their thanks and appreciation to the Plant Virology Research Center, School of Agriculture, Shiraz University, for the financial support.

Fig. 1. Symptoms of mosaic and chlorosis observed on the leaves of *Ruellia brittoniana* (A), Rudbeckia hirta (B), and *Dahlia* sp. (C). These plants were collected from green space in Shiraz City, Iran. The electrophoresis pattern of PCR fragments of approximately 350 bp in length amplified from the aforementioned symptomatic plants using a *Potyvirus* degenerate primer pair (Nib3R, Nib1) in RT-PCR (D) indicating that the tested plants have been infected with a potyvirus (In the subsequent tests, this virus was detected as potato virus Y). The 100 bp DNA Size Marker (M) from Denazist, Iran, was used for size reference.

Table 2. Accession numbers, and origin of some potato virus Y (PVY) isolates (isolated from potato, *Solanum. Tuberosum*) available in GenBank and a soybean mosaic virus (SMV) isolate (isolated from Soybean, *Glycine max*) used for phylogenetic comparison of PVY Nib gene nucleotide sequence in this study. Nucleotide (nt) identity percentage (%) of some PVY isolates available in GenBank with Iranian (IRSHZ) isolates of PVY from *Ruellia brittoniana***,** *Rudbeckia hirta*, and *Dahlia* sp. detected in this study, are shown.

PVY Isolate	Accession no.	Origin	nt identity % with IRSHZ isolate of PVY from *R. brittoniana*	nt identity % with IRSHZ isolate of PVY from *R. hirta*	nt identity% with IRSHZ isolate of PVY from *Dahlia* sp
NY100086	KY848013.1	USA	98.5	89.3	89.1
NY100001	KY848010.1	USA	98.5	89.6	89.4
ME_323_34	KY848051.1	USA	98.6	90.2	90.1
WI120092	KY848029.1	USA	98.8	91.1	91.1
ID_1258	KY847941.1	USA	98.7	91.8	91.6
ID-1_1_3A	KY847942.1	USA	98.5	92.3	92.3
PVY-OBR	AF255659.1	Brazil	98.5	92.5	92.1
Hco30	MH795852.1	Peru	98.3	93.8	93.5
O Dicol Capiro	MF176826.1	Colombia	98.1	94.3	94.6
P36	KX184816.1	Israel	94.8	98.2	97.3
ALYU-76	ON583980.1	Kazakhstan	94.5	98.3	98.4
NMG-3	MN607723.1	China	93.8	98.2	98.1
NMG-7	MN607725.1	China	92.7	98.2	98.1
G7A (SMV)	FJ640982	South korea	56.9	57.6	54.8

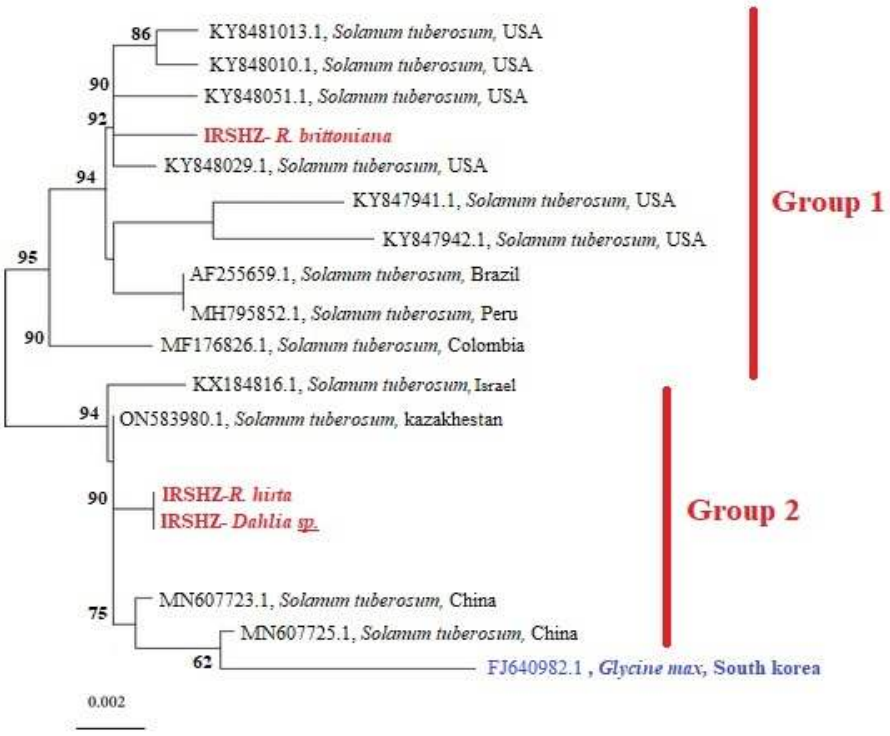

Fig. 2. A phylogenetic tree constructed based on the alignment of a 350 base pair fragment of the *RNA-dependent RNA polymerase* (*NIb*) gene of the genome of three Iranian-Shiraz (IRSHZ) isolates of potato virus Y (PVY) from *Ruellia brittoniana*, *Rudbeckia hirta* and *Dahlia* sp together with the same region of the homologous gene of some other PVY isolates available in GenBank from around the world (Table 1). Group 1 presents the Iranian isolate of PVY from *R. brittoniana* and the American continent PVY isolates and Group 2 presents the Iranian isolates of PVY from R. *hirta* and *Dahlia* sp. and Asian isolates of PVY. The phylogenetic tree was constructed using the maximum likelihood method and MEG A-8.0 Software. The tree was rooted with the Nib gene of strain G7A soybean mosaic virus (SMV) from South Korea (ACC. No. FJ640982), a member of the genus *Potyvirus*, which was used as an outgroup species (Table 1). Branches with less than 50% support were excluded from consideration. The details of the NCBI Accession numbers used in this study are provided in Table 1.

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تحقیقات کشاورزی ایران (۱۴۰۲) ۴۲(۱) ۴۷-۵۴

مقاله کوتاه

اولین گزارش از وقوع ویروس وای سیب‌زمینی در برخی از گیاهـان زینتی در ایران

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اطلاعات مقاله

تاریخچه مقاله:
تاریخ دریافت: ۱۴۰۲/۰۵/۱۱
تاریخ پذیرش: ۱۴۰۲/۰۸/۱۶
تاریخ دسترسی: ۱۴۰۲/۰۸/۲۰

واژه‌های کلیدی:
آغازگرهای عمومی پوتی‌ویروس
شیراز
فضای سبز
گیاهان زینتی

چکیده – طی بررسی‌های انجام شده از فضای سبز شهر شیراز در تابستان ۱۴۰۱، علائمی شامل موزاییک و کلروز روی برگ‌های گیاهان زینتی کوکب‌کوهی (*Rudbeckia hirta*)، یک گونه کوکب (*Dahlia sp.*) و پتونیای مکزیکی (*Ruellia brittoniana*) مشاهده شد. آر ان ای کل بطور جداگانه از برگ‌های ۱۰ نمونه‌ی دارای علائم و یک نمونه بدون علائم بعنوان کنترل منفی استخراج و با استفاده از یک جفت آغازگر دژنره پوتی‌ویروس‌ها (Nib2F, Nib3R) در واکنش رونویسی معکوس- زنجیره‌ای پلی‌مزار (RT-PCR) بکار رفت. RT-PCR منجر به تکثیر یک قطعه دی‌ان‌ای با اندازه مورد انتظار (حدود ۳۵۰ جفت‌باز) در تمام نمونه‌های دارای علائم شد، درحالی‌که هیچ قطعه‌ی دی ان ای از گیاه بدون علائم مورد آزمایش تکثیر نشد. ترادف نوکلئوتیدی به‌روش توالی‌یابی سنگر اندازه قطعه دی‌ان‌ای تکثیرشده را دقیقاً ۳۵۰ جفت‌باز تعیین و تأیید نمود که متعلق به ژن Nib ویروس وای سیب‌زمینی (PVY) است. مقایسه توالی نوکلئوتیدی آمپلیکون‌های جدایه‌های مورد مطالعه با توالی نوکلئوتیدی همان ناحیه از ژنوم برخی دیگراز جدایه‌های PVY موجود در بانک ژن نشان داد که جدایه PVY آلوده‌کننده پتونیای‌مکزیکی (*R. brittoniana*) بیشترین شباهت (۹۸/۸٪) را با جدایه‌ی PVY از آمریکا با رس‌شمار KY_848029.1 و جدایه‌های PVY کوکب‌کوهی (*R. hirta*) و یک گونه کوکب (*Dahlia* sp.) بیشترین شباهت (بترتیب ۹۸/۳٪ و ۹۸/۴) را با یک جدایه PVY از قزاقستان با رس شمار ON_583980.1 دارند.