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مقاله پژوهشی

آلودگی طبیعی نسرین (*Hippeastrum hybridum*) به ویروس کلروز فلفل و ویروس لکه زرد زنبق در ایران

حسین بیات[1*]، محمد حسین عظیمی[1] و سید مهدی بنی هاشمیان[2]

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چکیده

نسرین یا آماریلیس (*Hippeastrum hybridum*) متعلق به خانواده Amaryllidaceae است. گیاهان آماریلیس دارای نشانه‌های شبه ویروسی مانند لکه‌های حلقوی کلروتیک و الگوهای بیضوی متحدالمرکز در کلکسیون ژئوفیت‌های زینتی پژوهشکده گل و گیاهان زینتی، محلات، ایران مشاهده شد. تکثیر ژنوم با استفاده از آغازگرهای عمومی جنس *Orthotospovirus* و اختصاصی ویروس کلروز فلفل منجر به تکثیر قطعات مورد انتظار در برخی از نمونه‌های جمع‌آوری‌شده گردید. تعیین توالی قطعات تکثیر شده، آلودگی برخی از گیاهان مذکور را با ویروس کلروز فلفل و ویروس لکه زرد زنبق ثابت کرد. در هیچکدام از نمونه‌ها، آلودگی همزمان به این دو ویروس مشاهده نشد. این نخستین گزارش از حضور اعضای جنس *Orthotospovirus* بر روی گیاه نسرین یا آماریلیس از ایران است.

کلمات کلیدی: ایران، تشخیص، گیاهان زینتی، *Orthotospovirus*، RT-PCR

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[*] بخشی از یک پروژه ارایه شده به پژوهشکده گل و گیاهان زینتی، موسسه تحقیقات علوم باغبانی، سازمان تحقیقات آموزش و ترویج کشاورزی، محلات، ایران.

[**] مسئول مکاتبات، پست الکترونیکی: Bayat_new@yahoo.com

۱ استادیار و دانشیار پژوهشکده گل و گیاهان زینتی، موسسه تحقیقات علوم باغبانی، سازمان تحقیقات آموزش و ترویج کشاورزی

۲ دانشیار پژوهشکده مرکبات و میوه های نیمه گرمسیری، موسسه تحقیقات علوم باغبانی، سازمان تحقیقات، آموزش و ترویج کشاورزی

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Research Article

Natural infection of Hippeastrum hybridum with Capsicum chlorosis virus and Iris yellow spot virus in Iran

Hossein Bayat[1*]. Mohammad Hossein Azimi[1] . Seyed Mehdi Bani Hashemian[2]

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Abstract

Hippeastrum/amaryllis (*Hippeastrum hybridum* Hort.) belongs to *Amaryllidaceae*. Plants of the hippeastrum exhibiting virus-like symptoms of chlorotic ring spots and concentric oval patterns were found in the collection of ornamental geophytes of the Ornamental Plants Research Center, Mahallat, Iran. Amplification of the genome using general primers of the *Orthotospovirus* genus, and specific primers of capsicum chlorosis virus (CaCV) led to the amplification of the expected fragments in some of the collected samples. Sequencing of amplified fragments proved the infection of some of the samples with CaCV and iris yellow spot virus (IYSV). No simultaneous infection with these two viruses was observed in any of the samples. This is the first report of the occurrence of members of the genus *Orthotospovirus* on the hippeastrum plant from Iran.

Key Word: Diagnosis, Iran, Ornamental plants, Orthotospovirus, RT-PCR

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[**]Corresponding author: bayat_new@yahoo.com

[1] Assistant Professor Of Ornamental Plants Research Center(OPRC), Horticultural Sciences Research Institute(HSRI), Agricultural Research, Education And Extension organization(AREEO), Mahallat, Iran.

[2] Assistant Professor Of Citrus and Subtropical Fruits Research Center (CSFRT), Horticultural Science Research Institute (HSRI), Agricultural Research, Education and Extension Organization (AREEO),Ramsar, Iran.

Introduction

Hippeastrum, commonly known as amaryllis (*Hippeastrum hybridum* Hort.), is a genus from the family Amarylidaceae, and its endemic species are found in Brazil (Meerow *et al*. 1990). It is one of the bulbous ornamental plants and is among the top 20 flowers in the world. This plant is cultivated on a commercial scale in the Netherlands, South Africa, and the United States (Meerow *et al.* 1990). In recent years, efforts have been made to obtain new cultivars of this flower in Iran, which has led to the creation of promising genotypes based on morphological traits (Azimi & Karimi Alavijeh 2020). However, preparing healthy nuclear stocks from these superior genotypes is necessary for their preservation. Several viruses infecting naturally amaryllis have been reported worldwide. Hippeastrum mosaic virus (HiMV), Nerine latent virus (NeLV), Amazon lily mosaic virus (ALiMV), and Bean yellow mosaic virus (BYMV) belong to the genus *Potyvirus* (Alexandre *et al.* 2011, Malandraki *et al.* 2016, Raj *et al.* 2009, Xu *et al.* 2017), Hippeastrum latent virus (HiLV) a member of the genus *Carlavirus* (Chen *et al.* 2012a), Cucumber mosaic virus (CMV) from the genus *Cucumovirus*, Tobacco mosaic virus (TMV) belong to the genus *Tobamuvirus* (Dong *et al.* 2013) and Several members of the genus *Orthotospovirus* including Tomato spotted wilt virus (TSWV), Impatiens necrotic spot virus (INSV), Iris yellow spot virus (IYSV), Capsicum chlorosis virus (CaCV), and Hippeastrum chlorotic ringspot virus (HCSV) (Berniak 2016, Dong *et al.* 2013). Tospoviruses, belonging to the *Orthotospovirus* genus in the *Tospoviridae* family, cause significant economic loss in several crops and ornamental plants (Pappu *et al.* 2009). The use of molecular tools has been characterized tospoviruses into 30 species in five different serological groups (Karthikeyan *et al.* 2021). Tomato yellow ring virus (TYRV), IYSV, CaCV, TSWV, and INSV are among the viruses that were previously detected on crops and ornamental plants in Iran (Bayat *et al.* 2018, Beikzadeh *et al.* 2012, Ghotbi *et al.* 2005, Golnaraghi *et al.* 2007). CaCV belongs to the WSMoV serological group, and in Iran, was initially reported on *Rhudbeckia hirta* from Markazi province (Bayat *et al.* 2018). Before this, CaCV was reported from hippeastrum plants in India and Taiwan (Basavaraj *et al.* 2020, Chen *et al.* 2009). Hippeastrum isolate of CaCV from India showed most identities in nucleotide and amino acid of N gene sequences with Iranian isolate (Basarvaraj *et al.* 2020). IYSV in the IYSV serogroup, was first reported from onion in Iran from Khorasan Razavi province (Beikzadeh *et al.* 2012). Bayat and colleagues (2022) determined the molecular characteristics of the small genomic fragment of two onion and leek IYSV isolates from Markazi province.

In the present study, we report the incidence of members of the genus *Orthotospovirus* on the hippeastrum plant in Iran.

Materials and Methods

From 2020 to 2022, plants of the parents and promising genotypes of the hippeastrum/amaryllis exhibiting virus-like symptoms were observed in the collection of the Ornamental Plants Research Center, Mahallat, Markazi province, Iran. The symptoms of virus infection were observed in the form of prominent to medium or amorphous concentric chlorotic and circular chlorotic patterns on the collected plants (Figure 1). Total nucleic acid was extracted from symptomatic leaves of 10 plants using Masoomi-Aladizgeh and colleguse (2016) method. The DNA in the samples was removed using DNAse enzyme according to the manufacturer's instructions (Pars Toos Biotechnology, Iran). Reverse Transcriptase (RT) was carried out using the Easy cDNA Synthesis Kit (Pars Toos Biotechnology Company, Iran) according to the manufacturer's instructions at the temperature of 42 ºC for 60 minutes in a thermocycler (iCycler, Bio-Rad, USA). The PCR reaction was performed using the prepared Red Master Mix (Amplicon, Denmark) in a final volume of 15µL using universal orthotospovirus primers (Huang *et al.* 2018, Hasani-Mehraban *et al.* 2016) and CaCV-specific primers (Bayat *et al.* 2018). Purification of the amplified fragments from the gel was done using the GF-1 Gel DNA Recovery kit (Vivantis, Malaysia) according to the manufacturer's instructions. The fragments extracted from the gel were sequenced by Macrogene Company (South Korea). Sequences were analyzed using the BLAST with the available sequences in the GenBank and DNASTAR 5 software package (DNAStar Inc., Madison, WI, USA). Sequence alignment was performed in CLUSTAL W, and data were used as

input for the reconstruction of a phylogenetic tree using the Neighbor-joining method and bootstrap analysis with 1000 replication with MEGA X software (Kumar et al., 2018)

Fig.1 Symptoms of large chlorotic ring spots (a) caused by IYSV, concentric patterns (b) and amorphous chlorotic and necrotic spots (c) caused by CaCV on the hippeastrum leaves

Results and Discussion

The orthotospovirus universal primers dTospoF2 and dTopspoR2 (Huang *et al.* 2018), amplifying a fragment of about 300 base pairs corresponding to a part of the RNA-dependent RNA polymerase (RdRp) gene, caused the amplification of the expected fragment in all samples with viral symptoms including large chlorotic ringspot, amorphous chlorotic, and necrotic spots and concentric patterns. These results showed that the samples were infected with orthotospovirus species.

The general primer pair AS-EA-F andEA-R (Hasani-Mehraban *et al.* 2016), amplifying a part of the nucleocapsid gene of the Eurasian clade of orthotospoviruses caused the amplification of the expected fragment of 800 bp in two isolates of the hippeastrum plants. The use of a specific primer pair CaCV-UTR3-F and CaCV-N-R, amplifying the major part of the N gene of CaCV (Bayat *et al.* 2018) caused the amplification of a 900 bp fragment in eight plant samples infected with tospoviruses. No mixed infections of IYSV and CaCV were detected.

A 663 bp sequence amplified with general primer pair (AS-EA-F/EA-N) in two isolates Hip-DQ and Hip-17, shared 81.86-99.55% nucleotide identity with different IYSV isolates deposited in GenBank. Therefore, these two isolates belonged to the IYSV species, which was deposited in GenBank with the accession numbers OQ981190 and OQ981191, respectively, which shared 99.25% nucleotide identity.

The results of phylogenetic analysis based on the partial nucleotide sequence of the nucleocapsid gene of IYSV isolates of the hippeastrum plants showed that the Hip-DQ and Hip-17 isolates were closely related to the Iranian isolates of IYSV (Figure 2). This investigation showed that two IYSV hippeastrum isolates were more closely related to leek isolates (Le2M and Le1M) and onion isolates (O1D) from Markazi province than other Iranian isolates, and two other IYSV isolates from Markazi province (O2K and O1M) were in the other group (Figure 2).

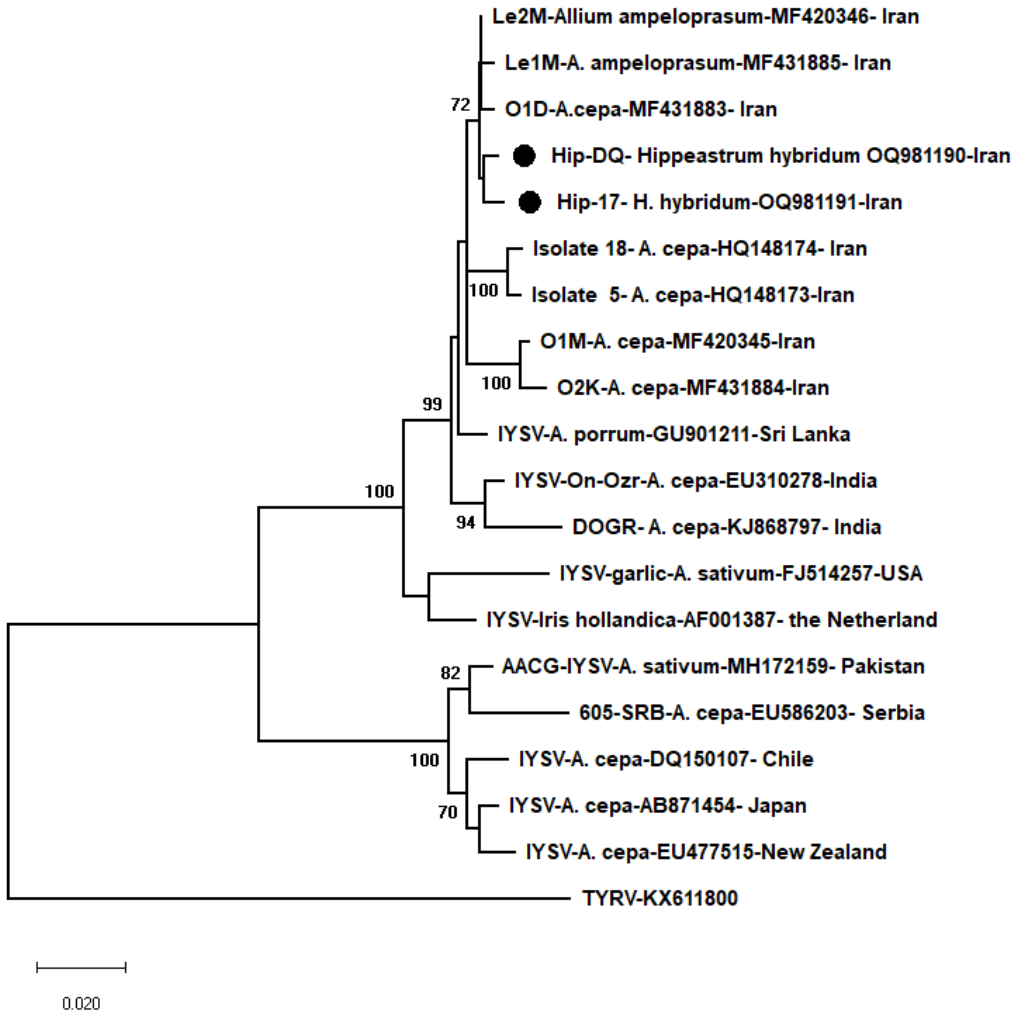

Fig.2 Phylogenetic relationships of Iris yellow spot virus isolates from the hippeastrum plants with other IYSV Isolates based partial nucleotide sequence of the nucleocapsid (N) gene. The tree was reconstructed based on the Neighbor-joining method with 1000 bootstrap replicates using MEGA X software. Bootstrap values less than 70% are not shown. Corresponding TYRV sequences were used as outgroups

The natural occurrence of IYSV on the hippeastrum /amaryllis plant is reported for the first time in this study, from Iran. Previously, IYSV was reported from the hippeastrum plant in Israel (Kritzman *et al.* 2001). The most important vector of this virus is the tobacco thrips (*Trips tabaci*), one of the most important pests of ornamental and crops in the Mahallat region. Considering the presence of the IYSV and its vector on hosts such as onion and leek (Bayat *et al.* 2022), which are mainly cultivated in the open fields of the region, it is far from expected that IYSV does not occur on the hippeastrum plants.

Nucleotide sequence 251 bp from the HP-102 isolate amplified with degenerate primer pair (dTospoF2/dTopspoR2), shared 94.02- 97.61% identity with different CaCV isolates in GenBank. Using CaCV-specific primer pairs, and observing the amplification of the expected fragment in this sample showed that this isolate belongs to the CaCV, which was deposited in GenBank with the accession number OR199889.

CaCV was reported for the first time from the rudbeckia plant in the West Asia region (Iran) by

Bayat *et al.* (2018). Before that, this virus was observed in many ornamentals and vegetables in different countries of East Asia (Chen *et al*. 2009, Kunkalikar *et al.* 2010, Sharma & Kulshrestha 2014, Zheng *et al*. 2011). This virus has been reported from the hippeastrum plants from India (Basavaraj *et al*. 2020), which showed the highest degree of similarity and identity in the nucleotide and amino acid sequence of the N gene with the Iranian isolate of CaCV. The nucleotide and amino acid sequences of the Indian CaCV isolate from the hippeastrum plant are very similar to the Iranian CaCV isolate (Basavaraj *et al*. 2020). Rudbeckia isolate and a recent isolate from the hippeastrum of CaCV were found from the same place at a very small distance, and it is likely that these two isolates are very similar to each other. CaCV transmission methods are vegetative propagation and different species of thrips. So far, three species of *Thrips palmi*, *Frankliniella schultzei* and *Microcephalothrips abdominalis* have been reported as CaCV vectors (Sharman et al., 2020). The presence of these species of thrips vector in Mahallat has not been proven. It is possible that one of these species or a new species of thrips is the vector of CaCV in the Mahallat. The high similarity between the hippeastrum isolates from India and the rudbeckia isolate of CaCV from Iran, it is possible that the virus entered Iran through the imported plant material of hippeastrum and then was transmitted to the rudbeckia plant through a biological vector. In this study, for the first time, the occurrence of some members of the genus *Orthotospovirus* on the hippeastrum plant was reported from Iran. Both species reported in this study were previously reported from the same region from other hosts. The epidemiology of CaCV in the Mahallat region is a subject that should be studied in future studies, considering its occurrence on two different hosts in recent years.

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