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Short Communication

First report of canna yellow streak virus in Iran

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ABSTRACT- *Canna indica* (canna) a species in the *Cannaceae* family, is a perennial ornamental plant widely used in landscape designing. Different species of plant viruses have been reported which infect this plant and act as a significant threat to canna plants, leading to a range of symptoms and a decrease in their decorative values, as well as lower quality propagated materials and significant financial losses. Canna cultivars that are extensively cultivated for their ornamental values are susceptible to a recently discovered *Potyvirus* known as canna yellow streak virus (CaYSV). This virus has infected many canna cultivars found in gardens, leading to significant impact and concern in horticultural settings. During a survey conducted in the summer of 2022, severe veinal chlorosis and veinal streaking symptoms were observed on the leaves of canna plants in the green space of Yazd City, Iran. Total genomic RNA was extracted from symptomatic leaves of 20 canna samples and one symptomless sample (negative control), and subjected to RT-PCR using a potyvirus degenerate primer pair (NIb3R, NIb2F). RT-PCR resulted in the amplification of a DNA fragment with the expected size of approximately 350 bp in all of the symptomatic samples, whereas no DNA fragment was obtained from a symptomless plant. The amplified DNA fragment was subjected to the Sanger sequencing method, and its size was confirmed to be exactly 350 bp. Sequence analysis of the nucleotide sequence of this amplicon with the same region of other corresponding CaYSV isolates in the GenBank revealed that the Iranian CaYSV isolate was the most similar (99.05%) to an isolate from Russia (Acc. No. MG_545919.1) and the less similar (89.59%) to a CaYSV isolate from United Kingdom (Acc. No. EF_466139.1).

INTRODUCTION

Canna indica a member of the *Cannaceae* family, is a popular perennial ornamental plant with attractive foliage and flowers that is widely used in landscape designing and is cultivated throughout tropical and temperate climates (Andrade-Mahecha et al., 2012). These plants are highly valued for their aesthetic properties, making them a significant part of the global horticulture industry (Beemster, 1982). Unfortunately, viruses pose a considerable threat to these plants, causing an array of symptoms and ultimately leading to reduced decorative value, lower quality propagated material, and severe financial losses. Due to vegetative propagation and the global trading of infected plant materials, viral diseases can have a particularly damaging effect on the economic value of canna plants (Kumari et al., 2021).

During the last two decades, seven viruses have been identified affecting canna plants, belonging to the *Potyviridae; e.g.*, canna yellow streak virus, (CaYSV), bean yellow mosaic virus (BYMV), and sugarcane mosaic virus (SCMV), *Cucumoviridae, e.g.*, cucumber mosaic virus (CMV), and tomato aspermy virus (TAV), *Tospoviridae, e,g.*, tomato spotted wilt virus (TSWV) and *Geminiviridae, e.g.*, canna yellow mottle virus (CaYMV) (Monger et al., 2007; Castillo et al., 1956; Li et al., 2019; Lockhart, 1988; Hollings and Stone, 1971; Yamashita et al., 1985; Rajakaruna et al., 2014; Kumari et al., 2021). Among the viruses that have been extensively studied in relation to canna plants, CaYSV, BYMV, and CaYMV are the most frequently documented (Chauhan et al., 2015; Zakubanskiy et al., 2017; Punsasi et al., 2015).

Recent studies have shown two new hosts for CaYSV in Hawaii and India, implying its expanded host range. Symptoms of plant viruses on canna-infected leaves are reported to be flecking, yellow mosaic, severe discoloration, leaf streaking, and necrosis along the veins, which in severe cases render plants unsalable (Kumari et al., 2021). Over the past few years, the canna plant has regained popularity as a garden plant; however, it has been reported that its growth has been accompanied by an increase in virus-related diseases (Li et al., 2019). This is mainly attributed to plant vegetative propagation (mainly through rhizome cuttings). The international trading of untested material

resulted in widespread virus issues among cultivated canna plants globally (Alexandre et al., 2017). Therefore, it is essential to scientifically identify the viral agents in canna plants.

During a survey in January 2022, in the green space of Yazd city *C. indica* plants were found to show severe yellow mosaic along the veins on the leaves (Fig. 1). A set of 20 symptomatic leaf samples were collected for analysis. Total RNA was extracted from these 20 symptomatic samples, along with an asymptomatic sample, using a Trizol reagent. (Denazist, Iran). The RNA samples were subjected to reverse transcription polymerase chain reaction (RT-PCR) using a degenerate primer pair including (NIb2F (5GTITGYGTIGAYGAYTTYAAYAA3), and NIb3R (5TCIACIACIGTIGAIGGYTGNCC3)) (Gibbs and Mackenzie 1997) targeting approximately a 350 nucleotide fragment in the Nib gene of the genome of potyviruses. PCR was carried out in a 25 µl of reaction mixture containing 12 µl PCR mastermix (Denazist, Iran), 9 µl H_2O, 2 µl of each primer (10 µM), and 1 µl cDNA. The mixture was denatured at 94°C for 5 minutes and amplified in 35 cycles of 94°C for 60 seconds (denaturing step), 54°C for 60 seconds (annealing step), and at 72°C for 60 seconds (extension step) with a final cycle at 72°C for 10 minutes (final extension). The PCR reactions were subjected to electrophoresis in a 1 % (w/v) tris-acetate agarose gel and staining with ethidium bromide.

The PCR products of the expected size (c. 350 bp) were obtained from all twenty samples. (Fig 1B), while there was no RT-PCR product found in the asymptomatic canna leaf sample. Amplified fragments of two samples were purified from the gel using a PCR purification kit (Qiagen. Co, Germany) and sequenced directly by Sinohe Inc. (Iran, Shiraz). DNA sequences were aligned and compared to other sequences in the GenBank using MEGA version V 8.0.

The identity of the obtained sequences was verified using the nucleotide blast (NCBI) search program. Multiple alignments of the nucleotide sequences were performed using the ClustalW program and compared with published related sequences available in GenBank.

The analysis of the resulting sequences showed that the Iranian canna tested isolate shares the maximum identity (99%) with a CaYSV isolate (ACC. No. MG545919.1) reported from Russia and the minimum identity (89.59%) with a united kingdom CaYSV isolate (ACC. No. EF466139.1).

Phylogenic trees obtained from the alignment of the partial genome nucleotide sequence (based on the 350 bp PCR product sequence), of an Iranian isolate of CaYSV (YZD-CA01)) and other CaYSV isolates available in GenBank (Fig. 2) with 100 bootstrap replicates using MEGA-8.0 Software and maximum likelihood method,(only bootstrap values of ≥34% were shown). The sequences of the same region of three maize dwarf mosaic virus (MDMV) isolates were used as outgroups. In the phylogenic tree, as can be seen (Fig. 2), the Iranian isolate of CaYSV (YZD-CA01)) was placed in a cluster together with the European isolates of CaYSV. Other CaYSV isolates constituted a clade that most closely related to included isolates of johnsongrass mosaic virus (JGMV, Fig. 2).

To identify potential co-infections of other viruses alongside CaYSV in the infected samples, PCR reactions were conducted using four different primer pairs. These included a specific primer pair (CMV CP-F, CMV CP-R) designed to amplify a fragment of CMV genome (Rizos et al., 1992), a degenerate primer pair for begomoviruses (primerB^C, Deng et al., 1994 / primer181^V, Rojas et al., 1993), and a degenerate primer pair for the *Tobamovirus* genus (TobamodF/TobamodR, Li et al., 2018). However, no PCR products were obtained using these primer pairs, indicating the absence of other viruses co-infections with CaYSV in the tested plants. Several different viruses from various taxonomic groups have been identified in canna in many countries across the globe, e.g., the UK, Belgium, the Netherlands, France, Italy, Austria, Israel, Kenya, India, Japan, Thailand, Brazil, the USA and Russia (Mitrofanova et al., 2018).

The CaYSV isolate found in Iran, known as CaYSV-YZD, probably shares similar effects on canna with other isolates of this virus, impacting the growth, development, yield, and aesthetic qualities of infected plants. It has been observed that vegetative propagation of canna plants contributes to the production of stable viral primary inoculum, which facilitates transmission by aphid vectors and subsequently accelerates the spread of CaYSV to healthy canna plants, as well as potentially other plant hosts (Mitrofanova et al., 2018). Given the economic significance of canna production and the susceptibility of various plant hosts to CaYSV in Iran, it is crucial to identify and comprehend the genetic diversity of viruses associated with canna plants. This knowledge is essential for accurate detection, improved control measures, and prevention of further infections.To our knowledge, this is the first report of the occurrence of CaYSV in Iran.

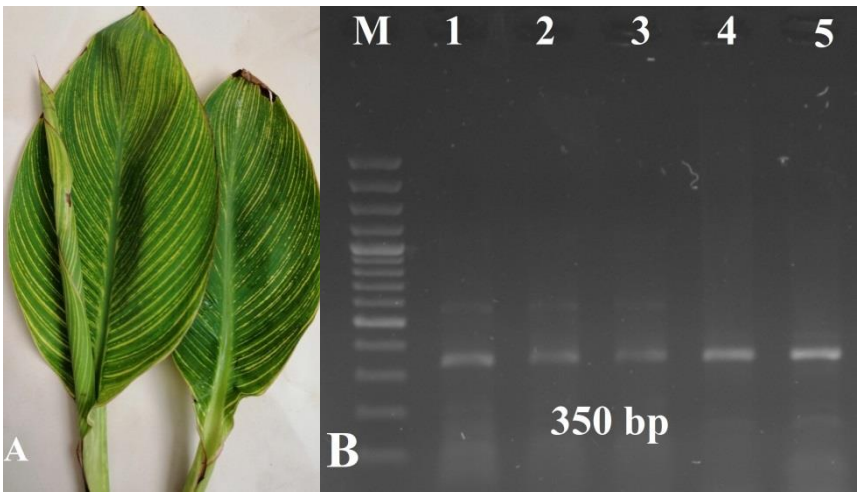

Fig. 1. A: Canna yellow streak symptoms observed on leaves of a *Canna indica* plant infected with CaYSV. The infected plant was collected in Yazd City, Iran. B: Electrophoresis pattern of amplified fragments approximately 350 bp length using degenerate primers (Nib3R, Nib1, see text) in RT-PCR from collected symptomatic canna plants indicating detection of CaYSV. (The 100 bp DNA Size Marker, Denazist, Iran).

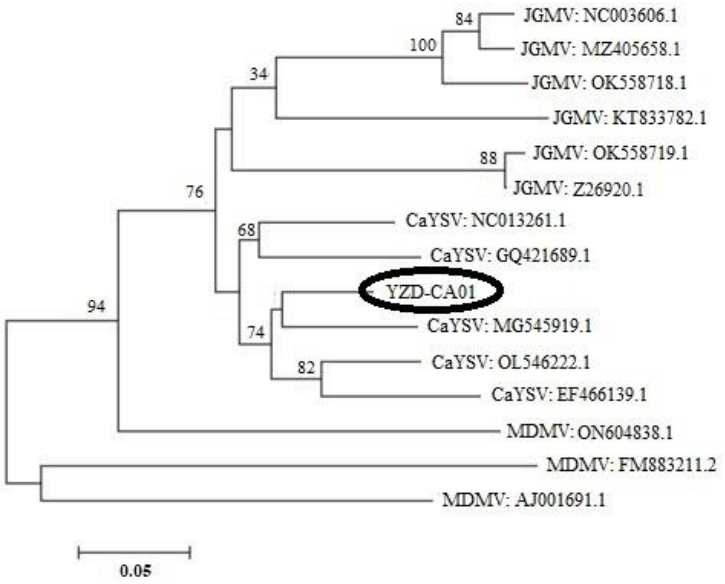

Fig. 2. The phylogenic tree that was drawn based on the PCR product sequence (350 bp) of a part of the genome of an Iranian isolate of canna yellow streak virus (CaYSV, YZD-CA01) compared with the sequences of the same region of CaYSV and Johnsongrass mosaic virus (JGMV) isolates genomes available in the GenBank. The accession numbers of the virus isolates that are shown in the phylogenic tree included NC003606.1, JGMV, complete genome; MZ405658.1, JGMV, isolate DSMZ PV-0803 Partial sequence; OK558718.1, JGMV isolate Ni1 polyprotein gene, complete cds; KT833782.1, JGMV isolate CNPGL, complete genome; OK558719.1, JGMV isolate O polyprotein gene, complete cds; Z26920.1, JGMV, protease 1 and 3, helper component 6K protein, coat protein, nuclear inclusion proteins; NC_013261.1, CaYSV, complete genome; GQ421689.1, CaYSV, complete genome, Nib polyprotein gene; MG545919.1, CaYSV, isolate KS, complete genome; OL546222.1, CaYSV, isolate GZ, complete genome; EF466139.1, CaYSV, isolate UK, polyprotein gene, partial cds; ON604838.1, MDMV, isolate DSMZ PV-0944, complete genome; FM883211.2, MDMV, isolate Sz0605, genomic RNA and AJ001691.1, MDMV, complete genome. The tree was reconstructed by the method of maximum likelihood and by MEGA-8.0 Software. The sequences of the same region of the genome of maize dwarf mosaic virus (MDMV) isolates from the genus *Potyvirus* were selected as out-group models. Branches with less than 34% support were not considered.

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چکیده – اختر (*Canna indica*) گونه‌ای از خانواده اختر (*Cannaceae*)، یک گیاه زینتی چندساله است که به‌طور گسترده در طراحی فضای سبز استفاده می‌شود. گونه های متفاوتی از ویروس‌های بیمارگر گیاهی این گیاه را آلوده نموده و به‌عنوان یک تهدید مهم برای گیاه اختر محسوب می‌شوند که منجر به طیف وسیعی از علائم در گیاهان اختر، کاهش ارزش کیفی زینتی آنها و همچنین مواد تکثیر شده با کیفیت پایین‌تر و منجر به خسارت مالی قابل‌توجهی می‌شوند. یک پوتی‌ویروس جدید به نام ویروس رگه زرد اختر (CaYSV) بسیاری از ارقام اختر را در فضای‌سبز که به دلیل ارزش زینتی خود به طور گسترده کشت می شوند آلوده می‌کند. این ویروس بسیاری از ارقام اختر را که در باغ ها یافت می شوند آلوده کرده است و منجر به نگرانی قابل توجهی در محیط های باغبانی شده است. طی بررسی انجام شده در تابستان ۱۴۰۱، علائم سبزردی شدید در امتداد رگبرگ‌های اختر در فضای‌سبز شهر یزد (ایران) مشاهده شد. آر ان ای کل از برگ‌های ۲۰ نمونه‌ی اختر دارای علائم و یک نمونه بدون علائم و به ظاهر سالم (کنترل منفی) استخراج و واکنش زنجیره‌ای پلی‌مزار معکوس (RT-PCR) با استفاده از یک جفت آغازگر دژنره پوتی‌ویروس‌ها (Nib2F, Nib3R) انجام شد. RT-PCR منجربه تکثیر یک قطعه دی ان ای با اندازه مورد انتظار (تقریبا باندازه ۳۵۰ جفت‌باز) در تمام نمونه‌های دارای علائم شد، درحالیکه هیچ قطعه دی ان ائی در گیاه بدون علائم مورد آزمایش تکثیر نشد. قطعه دی ان ای تکثیرشده به‌روش سنگر توالی‌یابی شدند و اندازه آن دقیقاً ۳۵۰ جفت باز تعیین شد. تجزیه و تحلیل BLASTn توالی نوکلئوتیدی جدایه‌های مورد نظر با سایر جدایه‌های CaYSV متناظر موجود در پایگاه ژنی نشان داد، جدایه یزد بیشترین شباهت (۹۹/۵٪) را با جدایه‌ای از روسیه با رس‌شمار MG545919.1 و کمترین شباهت (۸۹/۵۹٪) با جدایه‌ی CaYSV از انگلستان با رس‌شمار EF466139.1 دارد.