Shallot virus X: a hardly known pathogen of the genus Allium

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ABSTRACT

Crops belonging to the genus Allium, family Amaryllidaceae, are economically important and are widely cultivated around the globe. Some of the most problematic diseases of these crops are caused by members of three virus genera, Potyvirus, Carlavirus and Allexivirus. Shallot virus X (ShVX) is an Allexivirus that was first discovered in Russia in the nineties and it has since been described worldwide. The virus, transmitted mechanically or by the dry bulb mite (Aceria tulipae), affects virtually all members of the genus Allium and it causes yield reductions on these crops. ShVX is a positive-sense single-stranded monopartite RNA virus that contains six open reading frames (ORFs). The virus is mainly detected by RT-PCR but there are other serological and molecular techniques available for diagnosis. There are no methods described for managing crops infected by ShVX in the field, but tissue culture of meristems can render virus-free plants. Research on the ShVX-Allium pathosystem is needed for a comprehensive understanding of the physiological and molecular mechanisms used by the virus to infect its hosts and for developing methods for the effective control of viral infections.

Keywords: Allexivirus, ShVX, RT-PCR, onion, garlic.

RESUMEN

Los cultivos del género Allium, familia Amaryllidaceae, son económicamente importantes y ampliamente sembrados alrededor del mundo. Algunas de las enfermedades más problemáticas de estos cultivos son ocasionadas por virus de tres géneros, Potyvirus, Carlavirus y Allexivirus. Shallot virus X (ShVX) es un Allexivirus que fue descrito por primera vez en Rusia en la década de los noventa y desde entonces se ha descrito su presencia a nivel mundial. El virus, transmitido mecánicamente o por el eriófido de los bulbos (Aceria tulipae), afecta prácticamente a todos los miembros del género Allium ocasionando la disminución en la producción de estos cultivos. ShVX tiene un genoma monopartito representado por una cadena simple de ARN de polaridad positiva con seis marcos abiertos de lectura (ORFs). El virus se detecta principalmente por RT-PCR, pero existen otros métodos serológicos y moleculares disponibles para su diagnóstico. No existen métodos descritos para el manejo de plantas infectadas con ShVX en el campo, sin embargo se ha determinado que el cultivo de tejidos meristemáticos puede producir plantas libres de virus. Es necesario desarrollar investigaciones en el patosistema ShVX-Allium para lograr un entendimiento general sobre los mecanismos fisiológicos y moleculares que utiliza el virus para infectar a su hospedero y para desarrollar métodos para el control efectivo de las infecciones virales.

Palabras clave: *Allexivirus, ShVX, RT-PCR, cebolla, ajo.*

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INTRODUCTION

The genus *Allium,* family *Amaryllidaceae,* includes several plant species that are native to the northern hemisphere and are distributed through Europe, Asia, North America, and the north of Africa (Li *et al*., 2010; Phillips, 2010). Most *Amaryllidaceae* species grow in temperate zones, some can grow in the tropics and a few, like *A. schoenoprasum,* may grow in the arctic (Pastor and Valdes, 1983). Plants within this family are characterized by the uniformity of their floral structures despite the variability present in the morphology of other plant parts. Among the 2800 species belonging to the family *Amaryllidaceae,* only species within the *Allium* and *Asparagus* genera have agronomical importance (García *et al*., 2011). Onion (*A. cepa*), garlic (*A. sativum*), shallot (*A. cepa* var. *ascalonicum*) and leek (*A. ampeloprasum* var. *porrum*) are the most widely consumed *Allium* species worldwide (Rabinowitch, 2017). They have several medicinal uses, including treatment for diabetes, fever, jaundice, spleen enlargement, etc. (Sujitha *et al.,* 2016). In South America these crops are planted in tempered valleys and Andean territories with moderately cold climates, close to distribution centers (Ministerio de Agricultura, Ganadería y Pesca-MAGAP, 2013). In the Andean region, *Allium* crops are socio-economically important due to the direct or indirect workforce associated with their production.

Allium vegetables are the fourth most abundant group of commercially produced non-leguminous vegetables (FAO, 2016). The worldwide demand for bulb onion has been growing constantly showing a 70 % increase from 2000, when 50 million ton were produced, to 2014, when production reached 85 million ton (Boon, 2015). The leading exporters include The Netherlands, China, Mexico, India, USA, Egypt, Spain, New Zealand and Argentina. The main importers are USA, United Kingdom, Malaysia, Germany, Saudi Arabia, Japan, Canada, Czech Republic, Republic of Korea, and Brazil (Galmarini, 2018). United Kingdom and Germany are the main onion export market for Argentina and Chile, the largest exporters in South America, although their product also reaches United States, Canada, Japan and Malaysia. In countries like Peru and Chile, sweet onions are produced to export to the United States market from January to May. Onion and garlic production is economically important in other Latin American countries such as Mexico and Ecuador, where shallot production is also significant.

As a result of the great scale production and the vegetative nature of *Allium* propagation, pathogens that affect these crops abound. *Shallot virus X* (ShVX), from the *Flexiviridae* family and genus *Allexivirus,* is one of the several viruses infecting *Allium* (Howitt *et al.,* 2006; Zavriev and Vishnichenko, 2005). When infecting garlic, *Allexivirus* causes yield losses as the bulb size of infected plants is smaller compared to healthy plants (Cafrune *et al*., 2006).

DISTRIBUTION AND SYMPTOMS

Shallot virus X was firts reported in Russia (Vishnichenko *et al.,* 1993; Kanyuka *et al.,* 1992; Zavriev and Vishnichenko, 2005). The sequence contains six open reading frames (ORFs). The virus has also been reported in the Netherlands (van Dijk and van der Vlugt, 1994), India (Majumder *et al*., 2008), New Zealand (Perez-Egusquiza *et al*., 2009), Sudan (Hamed *et al.,* 2012), Italy (Taglienti *et al*., 2015) and Poland (Bereda and Paduch-Cichal, 2016). Recently, the virus was discovered in South America (Granda *et al.,* 2017), and it is presumed that it is distributed worldwide.

Plants infected by ShVX may be asymptomatic or display mild mosaic and chlorotic symptoms. In Argentina, a study showed a 14 to 32 % decrease in bulb weight and a 6 to 32 % decrease in bulb diameter, related to *Allexivirus* infection in garlic (Cafrune *et al*., 2006), resulting in significant yield losses. Nevertheless, it has been observed that, in natural conditions, viruses from the *Allexivirus, Carlavirus,* and *Potyvirus* genera can form viral complexes, making it difficult to relate symptomatology with a specific virus (Katis*et al.,* 2012). These viral complexes are common among plants from the *Amaryllidaceae* family (Mituti *et al*., 2015; Song *et al*., 1998).

TRANSMISSION

The only known vector of ShVX is the dry bulb mite (*Aceria tulipae*) (Van Dijk *et al.,* 1991; Sang Gu *et al*., 2007), a pathogen that can persist in plant material destined for propagation (Van Dijk *et al*., 1991; Adams *et al*., 2004). It has been observed that the pathogen employs unconventional routes to overcome the plant viral defenses, which may be related with a increase in the fitness of the virus (Arkhipov *et al*., 2017).

Mechanical sap inoculation has been proved effective for transmitting all members of the *Allexivirus* genus (Adams *et al*., 2004). In the other hand, virus transmission vectored by aphids has not been reported for any *Allexivirus* (Zavriev and Vishnichenko, 2005).

MORPHOLOGY AND GENOME

The first structural analysis of ShVX revealed similarities with the genomic organization of *Carlavirus* but lack ORF6 (Chen *et al*., 2004; Kanyuka *et al*., 1992). Later, as the number of available viral genomes increased, it was determined that ShVX together with other viruses detected in the genus *Allium* (*Garlic virus A, -B, -C, -D*) belongs to the genus *Allexivirus* (Song *et al.,* 1998; Sumi *et al, 1999*). Furthermore, comparative genome and protein analyses, indicate that the *Allexivirus* are part of the *Flexiviridae* family (Adams *et al*., 2004; Zavriev and Vishnichenko, 2005). The *Flexiviridae* includes viruses with filamentous virions, polyadenylated genome, and a triple gene block of movement proteins which are known to infect plants and plant-pathogenic fungi (Martelli *et al*., 2007).

Shallot virus X has a monopartite genome represented by a single strand positive RNA with six ORFs and a polyadenilated 3´-end (Kanyuka *et al*., 1992). The virions of ShVX are highly-flexible, filamentous particles of aproximately 800 nm of length and 12 nm of width, harbouring a 8890

nucleotide genome (Kanyuka *et al.,* 1992; Vishnichenko *et al.*, 1993). The virion length is similar to that of a potyvirus but its flexibility resembles that of a closterovirus (King *et al.,* 2012).

ORF1, located in the 5´-end of the ShVX genome, codes for a viral replicase of approximately 195 kDa. The proteins coded by ORF2 and ORF3, of 26 and 11 kDA, respectively (Kanyuka *et al.*, 1992), are similar to the triple gene block proteins, TGB1 and TGB2 that are found in diverse plant viruses (Morozov and Solovyev, 2003). ORF4 codes for P42, a serine rich 42 kDa-protein of unknown function and with no orthologous genes found in other *Allexivirus* nor in closely related genera, such as *Potexvirus* or *Carlavirus*. It is likely that P42 function is related to viral movement (Arkhipov *et al.*, 2013). ORF5 codes for the coat protein of 28 kDa, and ORF6 for a small, 15 kDa, cysteine-rich protein which is related to a small protein located close to the 3´end of *Carlavirus*. Lukhovitskaya *et al.*, 2014) demonstrated that this small protein acts as a viral transcription factor that supresses gene silencing.

Several filamentous and rod-shaped virus genomes contain a triple gene block that codes for three proteins that are essential for viral movement through plasmodesmata and systemic transport (Verchot-Lubicz *et al.*, 2010). Species from the genus *Allexivirus* contain part of the TGB3 within their genome but it is not expressed by an individual ORF, as it lacks a start codon (Kanyuka *et al.*, 1992; Chen *et al.*, 2001). Nevertheless, the TGB3 protein of ShVX and other *Allexivirus* may be expressed along with TGB2 from a bicistronic mRNA, requiring a leaky ribosome scanning (Lezzhov *et al.*, 2015).

DIAGNOSIS OF ShVX

ELISA (Enzyme-Linked Immuno Sorbent Assay) and PCR (Polymerase Chain Reaction) are the most common methods for plant pathogenic virus detection worldwide (Boonham *et al.*, 2014). Aditionally, the use of electron microscopy for morphological characterization and of indicator plants to determine alternative hosts and symptoms associated to viral infections, are common use in viral diagnosis. According to Boonham *et al.* (2014), the choice of diagnosis technique should depend on sensitivity, especificity, repeatibility and reproductibility.

Shallot virus X was first detected with indirect ELISA using horeradish peroxidase (Vishnichenko *et al.*, 1993). Currently, the use of double antibody sandwich ELISA, using ShVX-specific antiserum (DSMZ AS-1042) is common. Nevertheless, low viral titers present in plant samples impede a reproducible detection of the pathogen through ELISA (Ling *et al.*, 2001).

Traditional diagnostics methods based on serology, host range, and symptoms are limited in their ability to differentiate among *Allium* viruses, owing to the presence of complex viral mixtures exhibiting similar symptoms and possessing restricted host ranges. Reverse transcription coupled with the polymerase chain reaction (RT-PCR) is often used for the diagnosis of RNA viruses (Navot *et al.*, 1992). This is a fast, highly-sensitive method that, when multiplexed, allows the simultaneous identification of several different viruses (Crosslin and Hamlin, 2011). Methods for RT-PCR diagnosis of individual viruses affecting *Allium* species have been developed and optimized (Bereda and Paduch-Cichal, 2017; Dovas *et al.*, 2001; Perez-Egusquiza *et al.*, 2009; Vishnichenko *et al.*, 1993; Zavriev and Vishnichenko, 2005). However, it is necessary to effectively diagnose various types of *Allium* viruses at the same time. Multiplex detection assays have been developed for the detection of *Onion yellow dwarf virus* (OYDV) and *Shallot latent virus* (SLV)(Majumder*et al.*, 2008), OYDV, SLV, Garlic common latent virus (GarCLV) and allexiviruses in Indian garlic accessions (Majumder and Baranwal, 2014), and for detection of several garlic viruses (Hu *et al.*, 2015).

Recently, next-generation sequencing (NGS) of total RNA from infected plants have been widely adopted to identify all the different viruses that might be present in a sample (Pallás *et al.*, 2018). However, there are no reports about the use of NGS for detection of ShVX.

MANAGEMENT

Vegetatively propagated crops can be infected by different virus species that tend to accumulate over generations resulting in yield losses and varietal degeneration (Pagán *et al.*, 2014; Pramesh and Baranwal, 2015). Therefore, a periodical renovation, using virus-free plant material, is needed.

There is no literature available regarding methods to manage ShVX in the field; however, Perotto *et al.* (2010) and Shiboleth *et al.*(2001) reported that *Allexivirus*-free plants can be vegetatively reproduced using meristems. Garlic virus-free plants produced significantly higher yields

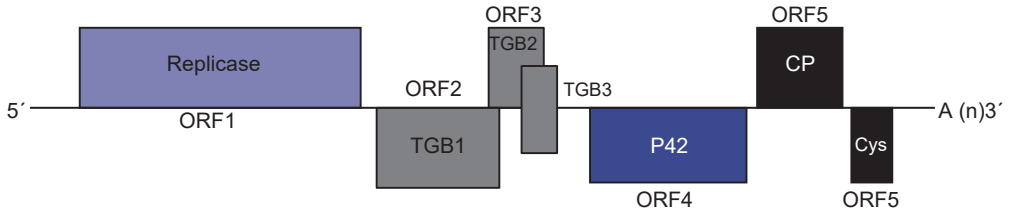

Figure 1. Genomic organization of *Shallot virus X*. TGB1, TGB2, and TGB3: triple gene block movement proteins; P42:protein of unknown function; CP: coat protein; Cys: cysteine-rich protein.

compared to infected plants. The stem-disc dome culture method and its combination with thermotherapy could also eliminate *Allexivirus* in garlic plants (Ayabe and Sumi 2001; Ghaemizadeh *et al.*, 2013).

CONCLUSION

Shallot virus X is a hardly known plant pathogenic virus that infects and causes yield reduction in *Allium* species. Since symptoms are unconspicuous and they are usually present in complex with other viruses, it is difficult to eliminate them. However, various procedures for controlling ShVX can be implemented depending upon situation. Development of resistant varieties, application of agronomic and biotechnological methods, and adequate plant nutrition and pest control, can be used in an integrated manner to manage this virus which is still a challenge for *Allium* producers.

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