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Unraveling Shallot Viral Diversity: PCR Detection and *In Vitro* Culture

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Unraveling Shallot Viral Diversity: PCR Detection and *In Vitro* Culture

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DEDICATION

To my parents for their guidance, unconditional love, and support to achieve each of my goals. To my grandparents for teaching me so many values, being perseverant and moving forward despite adversities. To my family and my boyfriend, for their infinite love and support, which allows me to pursue and achieve my dreams and goals.

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RESUMEN

La cebolla shallot (*Allium cepa* var. *aggregatum*) es una planta herbácea anual de la familia de las Amarilidáceas. Los clones de la cebolla chalote se cultivan en varias regiones del mundo, incluidos los huertos familiares de América, Europa y Asia. Sus cultivos tienen una importancia significativa en la gastronomía debido a su sabor único y propiedades nutricionales. Además, se la considera una fuente rica de fitoquímicos con algunos beneficios para la salud. En Ecuador, la chalote es considerada como un cultivo emergente que necesita encontrar soluciones para superar su susceptibilidad a fitovirus, particularmente de los géneros *Allexivirus, Carlavirus* y *Potyvirus*. Estos virus son patógenos importantes que repercuten negativamente en el rendimiento de los cultivos, provocando pérdidas económicas sustanciales y una disminución de la calidad de las cosechas en otros países. Las infecciones simultáneas por múltiples virus son comunes y pueden exacerbar sinérgicamente los daños en los cultivos. En este estudio, exploramos la presencia de los cinco virus más comunes que infectan a esta especie: virus latente del shallot (SLV), virus del enanismo amarillo de la cebolla (OYDV), virus X del shallot (ShVX), Virus de la franja amarilla del puerro (LYSV), y virus del mosaico amarillo de la cebolla (IYSV) en las chalotes de distintos proveedores de la ciudad de Quito, Ecuador. Mediante técnicas moleculares de RT-PCR, identificamos dos de estos virus: SLV, que permanece latente, e IYSV, caracterizado por una enfermedad sintomática grave. Para conseguir plantas libres de virus, realizamos cultivo *in vitro* de brotes apicales y obtuvimos el 100% de plantas de chalote libres del IYSV. Este estudio presenta la primera identificación del virus IYSV en las chalotes en el país, lo que podría incentivar a realizar un mejor manejo y monitoreo de patógenos en los cultivos, así como usar métodos eficientes de limpieza viral para obtener cultivos de mejor calidad y promover la producción y uso de esta variedad de cebolla.

Palabras clave: *Allium cepa* var. *aggregatum*, virus, detección molecular, brote apical, cultivo *in vitro*.

ABSTRACT

Shallot onion (*Allium cepa* var. *aggregatum*) is an annual herbaceous plant of the Amaryllidaceae family. Several regions worldwide, including home gardens across the Americas, Europe, and Asia, cultivate shallot clones. Shallot crops hold significant importance in gastronomy due to their unique flavor and nutritional properties, moreover; they are considered a rich source of valuable phyto-chemicals with some health benefits. In Ecuador, shallots are considered an emerging crop that needs to find solutions to overcome its susceptibility to s phyto-viruses, particularly from the *Allexivirus*, *Carlavirus*, and *Potyvirus* genera. These viruses are significant pathogens that impact negatively on crop yield, leading to substantial economic losses and a decline in crop quality in other countries. Simultaneous infections involving multiple viruses are common and can synergistically exacerbate crop damage. In this study, we explored the presence of five common viruses that infect shallots: Shallot latent virus (SLV), Onion yellow dwarf virus (OYDV), Shallot virus X (ShVX), Leek yellow stripe virus (LYSV), and Iris yellow spot virus (IYSV) in shallots from different suppliers in the city of Quito, Ecuador. We identified, using molecular RT-PCR techniques, two of these viruses: SLV, which remains latent, and IYSV, characterized by severe symptomatic disease. To obtain virus-free shallot plants, we performed shoot tip culture and achieved 100% IYSV-free shallot plants. This study presents the first identification of IYSV in shallots in the country, which could encourage better management and monitoring of pathogens in crops, as well as the use of efficient viral cleaning methods to obtain better quality crops and promote the production and use of this onion variety.

Keywords: *Allium cepa* var. *aggregatum*, viruses, molecular detection, shoot tips, *in vitro* culture.

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LITERATURE REVIEW

Shallot General Aspects

Shallot (*Allium cepa* var. *aggregatum*), a variety of onion, is an annual herbaceous plant of the Amaryllidaceae family. Shallot is a plant that can reach 20 to 30 cm in height. It has a compound bulb, cylindrical leaves, and adventitious fibrous roots. The stem grows 100 to 200 mm during the second year of plant life. The inflorescence is umbel-like and develops from the apical meristem. The outer leaf bases of the bulb lose moisture and become scaly, while the inner leaves thicken as the bulb develops (Pareek et al., 2017). The bulb usually weighs 5 to 25 grams, and the color varies depending on the variety (purple-green, red with brown, red with pink, yellow, gray, and white), its shape (rounded, spherical, and elongated), and its flavor, which depends on the area in which shallot is grown (Moldovan et al., 2022; Fitriana & Susandarini, 2019). Cultivated onions have two horticultural groups: the common onion that reproduces by seeds and the multiplying aggregatum group (shallot) with vegetative bulb reproduction and a short-grown cycle (Gupta et al., 2023). Most species grow in open, sunny, arid, and moderately humid climates. However, they have adapted to other ecological niches (Pareek et al., 2017). Some taxa tolerate alkali and saline environments and can grow in gravelly riverbanks, distinct types of forests, and subalpine wet meadows (Fritsch & Friesen, 2002). Shallot clones grow in various regions of the world, including America, Europe, and Asia. Larger-scale cultivation takes place in France, England, the Netherlands, Argentina, and Scandinavia, as well as in tropical regions such as Sri Lanka, West Africa, Thailand, and the Caribbean (Fritsch & Friesen, 2002). Each cultivar has different photoperiod, storage life, color, and bioactive compounds. Most cultivars are grown for salads, pickles, and dried bulbs (Pareek et al., 2017).

The importance of shallots worldwide and in Ecuador

Shallot is an important condiment in the gastronomy sector (Nhung & Quoc, 2024). It is associated with several traditional French recipes and is an authentic ingredient in Asian cuisine, such as red curry, green curry, Thai soup, and even some Indonesian dishes. Its bulbs are ingredients of haute cuisine due to the characteristic flavor and aroma produced by the organic sulfur compounds released through the enzymatic breakdown of flavor precursors found in its cells (Moldovan et al., 2022).

Shallots are a rich source of valuable phytochemicals such as fructans, flavonoids, and organosulfur compounds (antioxidant power) (Pareek et al., 2017). The sulfur and bioactive compounds have curative and prophylactic effects against various human ailments (Gargi et al., 2024). Furthermore, clinical trials and animal research studies have shown that shallot has health benefits, including treatment for viral infections, asthma, hypercholesterolemia, and osteoporosis, due to its antimicrobial, antiviral, analgesic, antipyretic, and anti-inflammatory properties (Moldovan et al., 2022; Nhung & Quoc, 2024; Pareek et al., 2017). Shallots are traditionally used to treat lipomas and onchocerciasis in some southern regions of Thailand and Nigeria (Moldovan et al., 2022).

The production of fresh shallots has experienced significant growth in recent years, with an increase in both local consumption and international trade. The countries with the highest consumption of onions and shallots (about 80%) in 2018 were China, India, and Pakistan (Moldovan et al., 2022). In 2022, there was an annual production of 110 and 4 million tons of dry and green shallots/onions, respectively (FAOSTAT, 2024). In Ecuador, the shallot is not registered as a different variety from the red onion; nevertheless, it is valued and used in the gastronomic sector, which makes it an emerging crop in the country, and the interest in its production is increasing (FAOSTAT, 2024; Heredia, 2016; Torres, 2014; Vega et al., 2015). Shallot is grown in the provinces of Carchi, Tungurahua, and Chimborazo. Shallots are also

imported specially from Peru. It is used mainly for self-consumption, for sale, and as an initial material for vegetative propagation (Heredia, 2016).

Viral infections in the *Allium* genus

Yield loss due to pests and diseases is common in shallot cultivation. Shallots are susceptible to several fungi, insect, and viruses (Rabinowitch & Kamenetsky, 2002). Viruses represent one of the primary pathogens impacting yield, resulting in significant economic losses and a reduction in crop quality. The viruses involved in infections belong to the *Allexivirus*, *Carlavirus*, and *Potyvirus* genera. Potyviruses are the most abundant and harmful, while carlaviruses and allexiviruses remain latent. Nevertheless, simultaneous infections involving various viruses are frequent and can synergistically exacerbate crop losses (Katis et al., 2012). The mechanism of viral entry and symptoms of infection vary depending on the type of virus, the host, the age of the plant, vectors, and environmental factors (Gunaeni et al., 2021).

A small number of potyviruses infect different types of onions and garlic**,** including leek yellow stripe virus **(LYSV**), onion yellow dwarf virus **(OYDV),** and shallot yellow stripe virus **(SYSV)**. The latter has negligible incidence, whereas LYSV and OYDV have an incidence rate of 45% to 73% in garlic and over 90% in onions from different countries. **OYDV** and **LYSV** are viruses transmitted by aphids in a non-persistent manner and not by seeds. Their virions are filamentous, with a length of 820 nm for the former, and 772 to 823 nm in length, 12 nm in width for the latter. *In vitro*, longevity is 3 to 4 days for LYSV and 2 to 3 days for OYDV. Its genome is linear single-stranded RNA in the positive sense and encodes for a polyprotein cleaved into ten mature proteins (Katis et al., 2012).

All RNA potyviruses replicate in the cytoplasm and the process is catalyzed by the viral replication complexes (VRC) bound to the cytoplasmic membrane (Shen et al., 2020). This virus initiates replication and silencing suppression at initial stages of infection. After entering

the cell, the RNA is translated into the cytoplasm. The expression strategy through polyprotein implies the production of amounts of all gene products and gives rise to large protein amounts that end up in inclusion bodies or are degraded. Mild mosaic symptoms in the host vary seasonally and may disappear soon after infection (López-Moya & García, 2008).

LYSV infects various *Allium* species. The symptoms of infection depend on the virus strain and host plant. It causes severe dwarf disease and reduces the bulb mass by up to 88%. Affected plants show chlorotic leaf stripping, dull stems, smaller sizes, and are less juicy. It can be transmitted by aphids, including *Myzus persicae, Aphis gossypii, Aphis pisum, Aphis fabae, Rhopalosiphum maidis, Schizaphis graminum, Rhopalosiphum padi,* etc. (Katis et al., 2012; Kawakubo et al., 2023). **OYDV** is a virus that can stunt plant growth. It causes yellow and chlorotic streaks on leaves, sagging and wrinkling, distortion in flower stalks, and a reduction in flower and seed numbers. Bulbs deteriorate and show premature sprouting, which makes plants more susceptible to low temperatures. Some studies indicate that it reduces bulb size and weight by up to 40% and seed production up to 50%. It is transmitted by over 50 aphid species, including *Myzus ascalonicus, Rhopalosiphum maidis, Acyrthosiphon pisum, and Myzus persicae* (Corrado et al., 2024; Katis et al., 2012).

SLV affects onions, shallots, garlic, and leeks. It is the first *Carlavirus* detected in *the Allium* species, and the host range is the *Alliaceae* subfamily. It is asymptomatic in onions, shallots, and garlic but acts synergistically with potyviruses. The virions are filamentous and measure 650 nm. The genome is linear single-stranded RNA in the positive sense, encoding six ORFs. The transmission can be by seeds, mechanical inoculation of plants, or aphids such as *Myzus ascalonicus, Myzus persicae, Neotoxoptera formosana, Aphis solani, and Aphis gossypii* (Katis et al., 2012; Pauzi et al., 2018).

SHVX is an A*llexivirus* that causes a reduction in crop yield and significant losses. Plants infected could be asymptomatic or show mild mosaic and chlorotic symptoms. ShVX is

a single-stranded, positive-sense RNA and consists of six ORFs. The genome contains a triple gene block that codes for three proteins essential for viral movement (TGB 1–3) (Kim et al., 2023). Also, the P42 protein is related to viral movement, while the cysteine-rich protein acts as a viral transcription factor that suppresses gene silencing (Jaramillo, 2020). Infected plants may be asymptomatic or show chlorosis and mosaic symptoms. Its effect is moderate compared with the potyvirus. A study in Argentina showed that bulb weight decreased by about 32%, as well as a reduction in diameter. It is transmitted mechanically or by dry bulb mites (*A. tulipae*). (Jaramillo, 2020; Y. Z. Wang et al., 2019).

IYSV is a tospovirus that infects onions, garlic, shallots, leeks, and chives. It is present in many countries worldwide, including Austria, Brazil, Germany, India, the United States, the Netherlands, Japan, South Africa, Peru, Mexico, and Chile. Viral particles are spherical, with a diameter ranging from 80 to 110 nm, and enveloped by a lipid membrane. It has a single-stranded tripartite RNA in the negative sense. It consists of large (L), medium (M), and small (S) portions. The L RNA encodes the RdRp protein responsible for the replication and transcription of the viral genome. The disease caused by IYSV is severe, causing yield losses and seed quality reductions. The most common symptoms are spindle-shaped, irregular, eye-shaped, or diamond-shaped lesions and chlorotic changes reflecting altered leaf chloroplast structure (Tripathi et al., 2015). It is not seed-borne, is not found in the bulbs of infected plants, and is transmitted exclusively by thrips (*Thrips tabaci*) (Katis et al., 2012).

Viral detection methods

Production of virus-free plants is necessary to control viral diseases and improve yield and quality of agricultural production while maintaining the genetic stability (Wang et al., 2018). Sensitive and reliable virus detection methods are essential in all certification systems and in monitoring and controlling *Allium* species. Certification ensures that propagation

material is free from quarantine pathogens, adhering to the requirements of each country and the standards of local and international markets (Varveri et al., 2015). Sometimes, plants appear to be virus-free and develop infection after an incubation period. Therefore, it is necessary to have virus detection methods available and affordable to test these plants (Hull, 2014). However, the diagnosis and characterization of viruses affecting the *Allium* genus have been restricted by a lack of appropriate and specific methods, resulting in misidentification due to symptoms caused by different viruses that sometimes overlap. Thus, identical or similar viruses have been described as distinct species and vice versa (Katis et al., 2012).

There are three main diagnostic methods available for detecting viruses in plants: bioassays, serological, and molecular (Varveri et al., 2015). Bioassays involve grafting plant material onto indicator plants. Symptoms typically develop over a long period and are common across all plants (Nie & Singh, 2017). Serological methods are based on the specific interaction between viral antigens and antibodies. The production of highly specific antisera against *Allium* plants is troublesome due to coinfections. The use of monoclonal antibodies has been used to overcome this problem and to differentiate between strains of potyviruses and carlaviruses by the variability of the N-terminal viral coat protein (CP) reaction (Lima et al., 2012). Although serology has been the main method for routine testing, serological cross-reactions can occur, and many host plants contain phenolic substances that precipitate proteins, making isolation of antigenically active viruses difficult or impossible (Lima et al., 2012; Matthews, 1967). Molecular methods have been optimized and developed for the large-scale testing of leaves and bulbs. Plant viruses infect and replicate mainly in leaves, which is visible by symptomatology and are similar in some viral infections (Hull, 2014b). Thus, specific molecular assay development, such as PCR and RT-PCR, allows a more reliable and sensitive diagnosis of viruses and the differentiation and characterization at the species level. This methodology

involves the development of primers, which leads to a wide range of detection and specificity (Katis et al., 2012).

In vitro culture and virus eradication

The vegetative propagation of shallot may result in increased pathogen accumulation and lead to low productivity (Pangestuti et al., 2023). Research indicates that virus infection reduces yield, bulb number, and weight by 45%. The viral infection in bulbs persists and is passed down through successive generations (Karjadi et al., 2023). Viral infection is a concern for growers because few effective treatment options are available to control the virus and vector (Tripathi et al., 2015).

Controlling viral infection in plants requires a comprehensive approach. This includes the use of certified virus-free seeds, controlling arthropod vectors, proper field management, removing infected plants, use of resistant varieties, use of insecticides, virus management during post-harvest, crop rotation, and surveillance and monitoring (Katis et al., 2012). In fact, the most effective way to control infection in *Allium* species is through the identification, development, and propagation of virus-free plants (Varveri et al., 2015). Various methods are available, including meristem culture, *in vitro* micrografting, virus-free seed production, chemotherapy, or cryotherapy. These methods have been used to improve the quantity and quality of the production of different onion varieties (Benke et al., 2023; Katis et al., 2012; Purnamasari et al., 2023; Varveri et al., 2015; Y. Z. Wang et al., 2019). The meristem culture is a plant tissue widely used to produce virus-free plants due to high genetic stability and growth potential, as the rate of meristem growth is higher than virus cell-to-cell transmission (Krishna et al., 2022; Vivek & Modgil, 2018). However, the success of elimination depends on the virus, the genotype of the plant species, and the treatment involved (Katis et al., 2012).

INTRODUCTION

Shallot (*Allium cepa* var. *aggregatum*), a variety of onion, is an annual herbaceous plant of the Amaryllidaceae family (Pareek et al., 2017). Shallot clones grow in several world regions, including home gardens in the Americas, Europe, and Asia (Fritsch & Friesen, 2002). This variety of onions belongs to the *aggregatum*, or multiplier group because it reproduces using vegetative bulbs (Leino et al., 2018). Each cultivar has differences in storage life, color, and bioactive compounds. Furthermore, shallots are a rich source of valuable phytochemicals such as fructans, flavonoids, and organosulfur compounds (antioxidant power) that have curative and prophylactic effects against various human ailments (Gargi et al., 2024; Pareek et al., 2017). Nevertheless, yield loss due to pests and diseases is common in bulb cultivation. Shallots are susceptible to several fungi, insects, and viruses. Viruses represent one of the primary pathogens impacting yield, resulting in significant economic losses and a reduction in crop quality (Katis et al., 2012; Rabinowitch & Kamenetsky, 2002). The main viruses involved in infections belong to the *Allexivirus*, *Carlavirus*, and *Potyvirus* genera. Simultaneous infections involving various viruses frequently exacerbate crop losses (Katis et al., 2012). In Ecuador, the shallot is not registered as a different variety from the red onion; however, it is valued and used in the gastronomic sector, which increases the interest in its production and can be considered an emerging crop in the country (Vega et al., 2015). Viral infection is a concern for growers because few effective detection and treatment options are available to control and monitor the virus and vector (Tripathi et al., 2015). Nonetheless, the success of elimination depends on the virus, the genotype of the plant species, and the treatment involved (Katis et al., 2012). The meristem culture is the most widely used method for the propagation of virus-free plants due to its high genetic stability and regeneration potential (Benke et al.,

2023; Katis et al., 2012; Krishna et al., 2022; Purnamasari et al., 2023; Varveri et al., 2015; Vivek & Modgil, 2018; Y. Z. Wang et al., 2019).

Viral detection and elimination are essential for healthy crop material production through vegetative propagation (Varveri et al., 2015). Therefore, the objectives of the present study were to identify five common viruses infecting shallots, including Shallot latent virus (SLV), Onion yellow dwarf Virus (OYDV), Shallot virus X (ShVX), Leek yellow stripe virus (LYSV), and Iris yellow spot virus (IYSV) (Katis et al., 2012; Ward et al., 2009) by molecular methods such as RT-PCR, and to evaluate the efficacy of shoot tip culture for the generation of virus-free shallots (*Allium cepa* var. *aggregatum*).

METHODOLOGY

Viral detection

Primer design

For viral detection, five viruses were selected for this study: OYDV, SLV, ShVX, IYSV, and LYSV. The nucleotide sequence of the target gene of each virus was searched on the NCBI database. For SLV, OYDV, and LYSV, the target gene selected was the coat protein, while for ShVX and IYSV, it was the replicase and nucleocapsid, respectively. All sequences were downloaded in FASTA format and inserted into the UGENE application to ensure that the sequences belonged to the specific virus and the region of interest. In this application, the sequences were aligned, and the consensus sequence was made.

In Primer3web (Untergasser et al., 2012), primer options were obtained for the consensus sequence and then validated on the PrimerBLAST (NCBI, 2024) and MFEprimer (Qu & Zhang, 2015) websites. After the validation process, the ideal candidates were selected, and degenerate primers were developed to amplify sequences that may have slight variations. All ten primer pairs underwent evaluation with positive controls and preliminary shallot samples, and the best five candidates (those that generate correct band size and the absence of unspecific bands) were chosen for further studies. For SLV (Majumder et al., 2008) and OYDV (Mahmoud et al., 2007), primers were chosen from literature, while for ShVX, IYSV, and LYSV, the designed primers were selected (Table 1).

Table 1. Primers and target genes that were used in the study.

Study	Virus	Oligo Name	Target gene	Primer (5' - 3')	Expected size
Majumder et al. (2008)	Shallot latent virus (SLV)	SLVLIT	Coat protein	GTGGTNTGGAATTAC	308 bp
				CAACATCGATTYTCTC	
Mahmoud et al. (2007).	Onion yellow dwarf virus (OYDV)	OYDVLIT	Coat protein	CGAAGCAAATTGCCAAGCAG	625 bp
				CGATTAGCTGCCCCTCTAAC	
Designed		ShVXLIT	Replicase	CCGTCGTGAATAGCCAAAGG	160 bp

	Shallot Virus X (ShVX)			GGTTCGAGGTTTGSTGARGA	
Designed	Iris yellow spot virus (IYSV)	IYSVDIS	Nucleocapsid	AAAATCTGCGGRCTTCCTCT TTTGCTGCCATGACTCTTGC	189 bp
Designed	Leek yellow stripe virus (LYSV)	LYSVDIS	Coat protein	ATGGCGCATTTYTCAGCAC GCTGCTTTCATTTGTGCRTG	221 bp

Development of positive controls

Five target sequences for each virus, including the nucleocapsid (IYSV), the replicase (ShVX), and the coat protein (SLV, OYDV, LYSV), were obtained from the NCBI database. From these sequences, small fragments that could be amplified by both types of primers (designed and literature-based primers) were selected and evaluated to avoid mismatches. Moreover, an *in-silico* analysis was performed to show that the plasmid sequence is not a factor of nonspecific amplifications. These sequences were sent to Macrogen (Seoul, South Korea) for insertion into pMG-Amp plasmids (ampicillin plasmid vector). The expected band sizes for positive controls for the viruses were the following: OYDV (625 bp), SLV (308 bp), ShVX (160 bp), IYSV (189 bp), and LYSV (221 bp).

Plant material for viral detection and *in vitro* culture

Shallot plants (*Allium cepa* var. *aggregatum*) were obtained from the Experimental Farm of the Universidad San Francisco de Quito (EF), an organic market called Organic Life (OM), and two suppliers from the Iñaquito market in Quito (Iq1-Iq2). The shallot plants were transported to the Plant Biotechnology Laboratory at USFQ. The whole plants (bulbs and leaves) were identified with a specific code, and their growth phases were documented photographically. The bulbs were removed from the leaves and stored at room temperature (18°C) for *in vitro* culture. Leaves were used for viral detection and were stored at -20°C for 24 hours and then at -80°C.

Viral detection by conventional PCR

RNA extraction from shallot leaf samples

Total RNA was extracted before and after *in vitro* culture using the TRIzol-chloroform protocol (Rio et al., 2010) (Supplementary Figure 1). Virus detection before *in vitro* culture was performed to identify the viruses present in the plant material, while the detection after shoot tip culture aimed to assess the efficiency of *in vitro* culture in eliminating the viruses. Before *in vitro* culture, RNA was extracted from 99 samples of shallot leaves: 23 from the Experimental Farm (EF), 26 from the Organic Life market (OM), and 25 from each supplier at the Iñaquito market-Quito (Iq1 and Iq2). After the second phase of *in vitro* culture, only 31 plantlets were assessed since they were obtained from shoot tips. The shallot leaves from these plantlets were used for molecular analysis: 11 from EF (called Trial 1), 8 from Eq1 (called Trial 2), and 12 from Eq3 (called Trial 3).

The shallot leaves were cut and immersed in liquid nitrogen. The frozen sample was pulverized by crushing the frozen tissue fragments in a previously cooled mortar. The 200 to 300 mg of fine powder was placed in a 5 mL Eppendorf tube, and 1 mL of TRIzol was added to each sample. After 5 minutes at room temperature, 0.2 mL of chloroform was added and mixed vigorously by hand for 15 seconds. The samples were kept for 2 to 3 minutes at room temperature and then centrifuged at maximum speed (4°C) for 15 minutes until two phases were observed: an upper phase containing RNA and a reddish lower phase containing proteins and DNA. The upper phase was transferred to a clean tube, taking care not to touch the lower phase. Subsequently, 0.5 ml of cold isopropanol was added for each milliliter of the upper phase, mixed by hand, let to stand for 10 min, and centrifuged at 10000 g for 10 min (4°C). All supernatant was removed by pouring (not pipetting), and the pellet was washed twice with 1 ml of 75% ethanol. Finally, the pellet was dried for 5 min, ensuring complete removal of ethanol droplets, and the RNA was resuspended with 50 µL of DEPC water. The RNA was left at 4°C

for 24 hours and then stored at -20°C. RNA quantity and purity, as well as integrity, were checked by Nanodrop and Qubit.

cDNA synthesis

The cDNA was synthesized using SuperScript III (Invitrogen). The total RNA obtained from samples was run along with random hexamer primers (Thermo Fisher Scientific) and carried out in a 20 µL reaction mixture containing 1 µL of random hexamer primers (Thermo Fisher Scientific), 1 µL of dNTPs [10 mM], 11 µL of a mix of RNA and water, 4 µL of Thermo buffer [10x], 1 µL of DTT, 1.25 µL of PCR water, and 0.75 µL of SuperScript III (Invitrogen). The thermal cycling conditions included a pre-incubation step of 65°C for 5 min followed by 30 cycles at 50°C for 60 min, 47 to 67°C for 15 min, and 70°C for 15 min. The cDNA samples were stored at -20°C).

Reverse transcription polymerase chain reaction (RT-PCR)

RT-PCR was conducted using cDNA as the template, along with five different virus-specific primers in equimolar concentrations. Positive and negative controls were used to make sure that the PCR was carried out correctly. RT-PCR reactions to detect viruses in the shallot samples were performed in a final volume of 25 µL, which contained 0,75 µL of $MgCl_2$ [50 mM], 2.5 µL of Buffer [10x], 0,5 µL of primers for each virus: SLV, OYDV, ShVX, LYSV, and IYSV [10 µM], 0,5 µL of dNTPs [10 mM], 0.2 µL of Taq Platinum, and 2 µL of DNA. Thermal profile conditions were 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 58°C (OYDV, lYSV), 47°C (SLV), 61,5°C (ShVX, IYSV) for 30 s, 72°C for 1 min, and finally an extension of 72°C for 3 min. The RT-PCR products were visualized by 1.5% agarose gel electrophoresis, stained with SYBR Safe, and visualized under UV light on a photo-documenter (BIO-RAD). The sizes of the expected PCR products were: SLVLIT (308 bp), SLVDIS (215 bp), OYDVLIT (625 bp), OYDVDIS (170 bp), SHVXDIS1 (160 pb), SHVXDIS2 (221 bp), IYSVLIT (401 bp), IYSVDIS (189 bp), LYSVLIT (126 bp), LYSVDIS (221 bp).

In vitro culture

The protocol of *in vitro* culture used in this study was standardized and taken as a based protocols such as Ramírez (2012) and Vega (2015), with some modifications (Supplementary Table 1). These modifications included using fresh bulbs without a drying or cooling period, applying a fungicidal wash, using Mertect (Syngenta) fungicide instead of Benomyl (Agripac), and omitting the fungicide from all culture media (Figure 1).

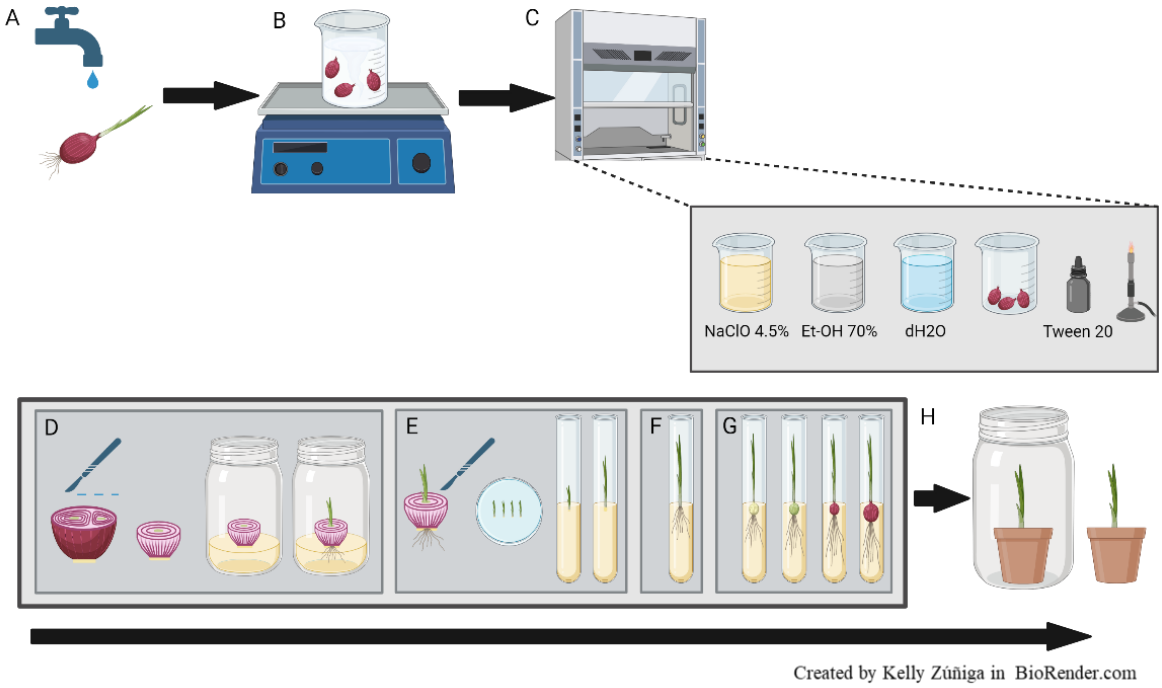


Figure 1. Scheme of shallot shoot regeneration from shoot tips. **A.** Washing bulbs with running water. **B.** Washing bulbs with Mertect fungicide (15 minutes). **C.** Disinfection protocol with ET-OH 70%, NaClO 4.5% and Tween 20 inside the laminar flow chamber. **D.** Culture of basal discs (M1 medium). **E.** Shoot tip culture (M1medium). **F.** Rooting (M2 medium). **G**. Bulb formation (M3 medium). **H.** Acclimatization of shallots.

First phase: Disinfection of plant material

In this study, a total of 73 shallot bulb samples were analyzed: 23 from EF (Trial 1), 25 from Eq1 (Trial 2), and 25 from Eq2 (Trial 3). Samples from the OM could not be used because of contamination. Shallot bulbs were divided into control and treatment groups. In Trial 1, 5 control samples and 18 treatment samples were selected, while in Trials 2 and 3, 5 control

samples and 20 treatment samples were used. The differences between the groups were the washing in Mertect fungicide (no washing in the control and 15 minutes of washing in the treatment) and the time that bulbs were immersed in sodium hypochlorite (30 minutes to control, and 35 minutes to treatment) (Supplementary Table 1). The disinfection protocol started when bulbs were pre-washed in tap water for 10 minutes to remove the remains of the soil and discard the dry layers. The bulbs that belong to the treatment group were immersed in Mertect fungicide (3.5 g/L) and shaken at 420 rpm for 15 minutes. Control bulbs were not immersed in the fungicide.

Afterwards, in a laminar flow chamber (LABCONCO Purifier Clean Bench), bulbs were immersed in ethanol (70%) for 10 minutes. The remaining ethanol was removed, and then the bulbs were submerged in sodium hypochlorite (4.5%) with three drops of Tween 20 for 30 minutes for the control group and 35 minutes for the treatment group. Later, the bulbs were rinsed with sterile distilled water. One-third of the length of the bulb was excised with a sterile scalpel, retaining the basal portion, and removing external cataphylls to obtain an explant called basal disc of 1.5 cm in diameter and 1 cm in height. Each explant was cultured on M1 initial medium in a glass bottle (Supplementary Table 2).

Second phase: Shoot tips culture

After 15 days in culture, once the sterile basal discs germinated to produce plants, shoot tips (0.5 to 5mm) contained the meristem (0.1 to 0.5 mm) along with the first leaf primordia (Nehra & Kartha, 1994; Wang et al., 2020) were excised and placed in glass tubes with 25 ml of M1 regeneration medium (Supplementary Figure 2). After one month of shoot tip culture, young leaves were collected from each plantlet for the virus detection. All plantlets were subcultured on M2 rooting medium (Supplementary Table 2). After four weeks, when plantlets developed roots, they were transferred to M3 medium every 30 days (Supplementary Table 2).

All plant material was cultured in the culture room at 25 ± 1 °C with a photoperiod of 16 hours in white light.

Acclimatization

After eight months of *in vitro* culture, when plants showed roots and bulbs, acclimatization took place. The shallot plants were removed from the tube and washed with distilled water to remove the agar. They were dried, planted in clay pots with autoclaved black soil and placed inside 1,900-mL glass jars. The flasks were covered with plastic wrap, fastened with a rubber band, and kept in the culture room at 25 °C with a photoperiod of 16 hours. Twice a week, plants were watered with sterile distilled water, and two holes were made in the plastic wrap. After one month, the plastic was completely removed. All plants received Hoagland fertilizer (Rajan et al., 2019) once every 15 days.

Data collection and statistical analysis

The efficiency of the disinfection protocol employed was assessed by measuring variables such as the percentage of sterility and the sprouting of the basal discs after 15 days of culture. Additionally, the efficiency of the virus elimination was evaluated through the shoot tip culture. We also monitored the percentages of sprouting, plantlet development, rooting, and bulb production over 300 days of *in vitro* culture. Statistical analysis was conducted using the Kruskal-Wallis test for sterility, plantlet development, rooting, and bulb formation; the Mann-Whitney test to establish differences between control and treatments in sterility of basal disc; and the Analysis of Variance (ANOVA) to see if there are differences between the shoot tip averages obtained from each basal disc from each shallot source. All statistical analyses were performed using Prism 5.03 for Windows (GraphPad Software, San Diego, CA, USA). Statistical significance was considered at a significance level of $p < 0.05$.

RESULTS

Viral detection

Primer selection

The DNA primers for the viruses SLV, OYDV, ShVX, LYSV, and IYSV were evaluated by PCR and agarose gel electrophoresis. Two types of primers were tested for each virus: primers designed in this research (amplifying for virus coat protein, replicase, and nucleocapsid) and primers taken from the literature (Table 1) (Mahmoud et al., 2007; Majumder et al., 2008). The primer selection was based on PCR products in agarose gel electrophoresis (correct band size and absence of nonspecific bands). All ten primers showed amplification with positive controls (Supplementary Figure 3A-J). Nine bands showed the expected DNA band size of the controls tested, except with the OYDVDIS primer, which produced a band of unexpected size (Supplementary Figure 3D). Several PCRs were performed to evaluate amplification (Supplementary Figure 4). There was amplification for SLV with both SLV primers. However, the SLVDIS primer showed nonspecific bands (Supplementary Figure 4B), so SLVLIT was selected for further analysis. Finally, analyzing the PCR results with controls and preliminary shallot samples, literature-based primers were selected for SLV and OYDV, and designed primers were chosen for ShVX, IYSV, and LYSV for future molecular analysis.

Total RNA extraction from shallot leaves

Total RNA extraction from the 99 samples of shallot leaves analyzed (23 from EF, 26 from OM, and 25 from each supplier Iq1 and Iq2) before *in vitro* culture showed the following RNA concentration average: 552.2 ng/µL for EF individuals; 508.20 ng/µL for OM; 493.8 ng/µL for Iq1; and 261.4 ng/µL for Iq2. The 260nm/280nm absorbance index was 1.97±0.04; while the 260nm/230nm index was 1.47±0.4 (Supplementary Table 3). After *in vitro* culture, 31 samples of shallot plantlets leaves were selected for analysis (11 from Trial 1, 8 from Trial 2, and 12 from Trial 3, Total RNA concentration of 691.30 ng/µL were achieved for Trial 1;

910.50 ng/µL for Trial 2; and 722.50 ng/µL for Trial 3. The 260nm/280nm absorbance index was 1.97±0.05; while the 260nm/230nm index was 2.08±0.3 (Supplementary Table 4).

Viral detection before in *vitro culture*

The established cDNA and PCR protocols worked correctly and yielded PCR products, which were visualized by agarose gel electrophoresis. The results indicated that of the 99 plants evaluated before *in vitro* culture, a total of 87 samples were positive for SLV; 29 samples were positive for IYSV; 27 samples presented both viruses, which is considered a co-infection; and 10 samples assessed negative for both viruses. In addition, it can be noted that 72% of the Iq2 while the 16% of Iq1 plants were coinfected with IYSV. All samples showed negative amplification for OYDV, ShVX, and LYSV (Table 2).

Table 2. Virus detection in shallot plants before *in vitro* culture

Virus detection													
Source	**Plants analyzed (n)**	**One virus**				**Coinfection**		**SLV and IYSV Negative**		**Total SLV positive**		**Total IYSV positive**	
		SLV		**IYSV**		**SLV and IYSV**							
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Experimental farm	23	23	100,00	0	0,00	0	0,00	0	0,00	23	100,00	0	0,00
Organic market	26	21	80,77	0	0,00	5	19,23	0	0,00	26	100,00	5	19,23
Iñaquito market 1	25	10	40,00	2	8,00	4	16,00	9	36,00	14	56,00	6	24,00
Iñaquito market 2	25	6	24,00	0	0,00	18	72,00	1	4,00	24	96,00	18	72,00
Total	**99**	**60**	**60,61**	**2**	**2,02**	**27**	**27,27%**	**10**	**10,10**	**87**	**87,88**	**29**	**29,29**

*Virus detection, number of positively infected samples to the total of samples subjected to virus detection.

Viral detection after in *vitro culture*

After the shoot tip culture, 31 plants were analyzed for viral detection (11 from Trial 1, 8 from Trial 2, and 12 from Trial 3) (Tables 3-5). The *in vitro* culture of shoot tips successfully eliminated IYSV but not SLV, which was still present in all shallot samples assessed (Tables

3-5). In addition, four samples were negative for SLV before *in vitro* culture but tested positive after this process (Tables 4-5).

Table 3. Virus detection results in shallots from the experimental farm (Trial 1), before and after *in vitro* culture.

Sample	Virus			
	SLV		IYSV	
	Before	After	Before	After
1	+	+	-	-
2	+	+	-	-
3	+	+	-	-
4	+	+	-	-
5	+	+	-	-
6	+	+	-	-
7	+	+	-	-
8	+	+	-	-
9	+	+	-	-
10	+	+	-	-
11	+	+	-	-

Abbreviation: IND, Individual; SLV, Shallot Latent Virus; IYSV, Iris Yellow Spot Virus.

Table 4. Virus detection results in shallots from Iñaquito 1 (Trial 2), before and after *in vitro* culture.

Sample	Virus			
	SLV		IYSV	
	Before	After	Before	After
1	+	+	+	-
2	-	+*	-	-
3	+	+	-	-
4	-	+*	+	-
5	+	+	-	-
6	-	+*	-	-
7	+	+	+	-
8	+	+	-	-

Abbreviation: IND, Individual; SLV, Shallot Latent Virus; IYSV, Iris Yellow Spot Virus.
*Samples that tested negative before and positive after the disinfection protocol.

Table 5. Virus detection results in shallots from Iñaquito 2 (Trial 3), before and after *in vitro* culture.

Sample	Virus			
	SLV LIT		IYSV DIS	
	Before	After	Before	After

1	+	+	-	-
2	+	+	+	-
3	+	+	+	-
4	+	+	+	-
5	+	+	+	-
6	+	+	+	-
7	+	-	-	-
8	+	+	+	-
9	-	+*	-	-
10	+	+	+	-
11	+	+	+	-
12	+	+	+	-

Abbreviation: IND, Individual; SLV, Shallot Latent Virus; IYSV, Iris Yellow Spot Virus.
*Samples that tested negative before and positive after the disinfection protocol.

In vitro culture

First phase: Disinfection of plant material

The disinfection protocol of basal discs resulted in a sterility rate of 44.40% for Trial 1 and 35% for trials 2 and 3 (Table 6). Statistical analysis showed no significant difference in the sterility of the basal discs among the three trials (p-value = 0.7937), indicating the reproducibility of the sterilization process. Comparing the control and treatment group, only Trial 3 showed a statistically significant difference in sterility (p-value = 0.0149) (Table 5).

Table 6. Sterility rates of basal discs of shallots after 15 days of *in vitro* culture.

Trial	Source	Esterility (%)			
		Control		Treatment	
1	**EF**	3 of 5	40%	8 of 18	44,40%
2	**Iq1**	1 of 5	20%	7 of 20	35%
3	**Iq2**	5 of 5	100%	7 of 20	35%
Mean		**9 of 15**	**53,30%**	**22 of 58**	**38,13%**

Abbreviation: EF, Experimental Farm; Iq1, Iñaquito 1; Iq2, Iñaquito 2. **Control:** Washing with tap water, no washing with fungicide, Et-OH (70%) for 10 min, NaClO (4,5%) + Tween-20 for 30 min, Washes with sterile distilled water (dH_2O). **Treatment:** Washing with tap water, washing with Mertect fungicide (3,5 g/L) for 15 minutes, Et-OH (70%) for 10 min, NaClO (4,5%) + Tween-20 for 35 min, Washes with sterile distilled water (dH_2O).

Sprouting of basal discs

Sprouting of basal discs was 100% in all assays and controls after 15 days of *in vitro* culture (Table 7). However, analysis of shoots over time revealed faster growth in the treatment group compared to bulbs from the control group (Supplementary Figure 5).

Table 7. Sprouting rates of shallot basal discs after 15 days of *in vitro* culture.

Trial	Source	Sprouting (n,%)			
		Control		Treatment	
1	**EF**	5 of 5	100%	18 of 18	100%
2	**Iq1**	5 of 5	100%	20 of 20	100%
3	**Iq2**	5 of 5	100%	20 of 20	100%
Mean		**15 of 15**	**100%**	**58 of 58**	**100%**

Abbreviation: EF, Experimental Farm; Iq1, Iñaquito 1; Iq2, Iñaquito 2. **Control:** Washing with tap water, no washing with fungicide, Et-OH (70%) for 10 min, NaClO (4,5%) + Tween-20 for 30 min, Washes with sterile distilled water (dH_2O). **Treatment:** Washing with tap water, washing with Mertect fungicide (3,5 g/L) for 15 minutes, Et-OH (70%) for 10 min, NaClO (4,5%) + Tween-20 for 35 min, Washes with sterile distilled water (dH_2O).

Second phase: Shoot tip culture

Plantlets development, rooting and bulb formation.

The average number of shoot tips obtained from shoots that germinated from each basal disc was 5.250 for Trial 1, 3.14 for Trial 2, and 2.85 for Trial 3 (Figure 2). These data do not show statistically significant differences (p-value = 0.0618). The percentage of plantlet development from the shoot tip was highest in Trial 1 (84.31%), with the lowest percentage in Trial 2 (57.9%) (Table 8). These percentages are statistically significant between the trials (p-value = 0.0006). Plantlets grew up to plants and all plants within each trial were successfully rooted, with no statistically significant differences (p-value = 0.2419). Regarding bulb formation, in Trial 1, 100% bulb formation was obtained after 300 days of culture, while in Trials 2 and 3, between 82% and 92% of the plants developed bulbs at the same time (p-value = 0.0001).

Table 8. Percentages of plantlet development, rooting and bulb formation.

Trial	Source	Shoot tips	Plantlets	
		Plantlets development (n; %)	**Rooting (n; %)**	**Bulb formation (n; %)**
1	EF	43 of 51; 84,31%	48 of 48; 100%	48 of 48; 100%
2	Iq1	22 of 38; 57,9%	26 of 26; 100%	24 of 26; 92,3%
3	Iq2	20 of 32; 62,5%	17 of 17; 100%	14 of 17; 82,35%

Note: Bulb formation data up to 300 days of culture.

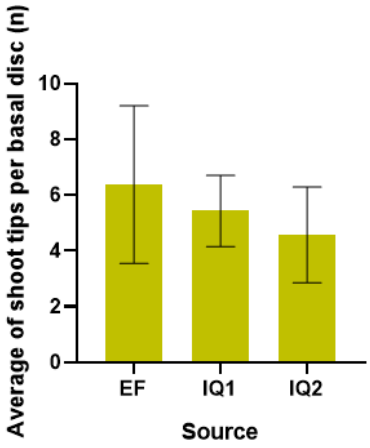


Figure 2. The average number of shoots tips extracted per basal disc.

Abbreviation: EF, Experimental Farm; Iq1, Iñaquito 1; Iq2, Iñaquito 2 (p-value = 0.0618).

After 60 days of shoot tip culture, approximately 50% of the plants in Trials 1 and 2 developed roots, while in Trial 3 only 46.2% (Figure 3). Bulb formation was found to be faster in Trial 1, where plants started to develop bulbs after 90 days of culture, while in Trials 2 and 3, bulb development occurred after 210 days of culture. All plants developed bulbs during 300 days of culture in Trial 1, while Trials 2 and 3 at 360 days of culture (Figure 4). All plants that showed root and bulb formation were acclimatized.

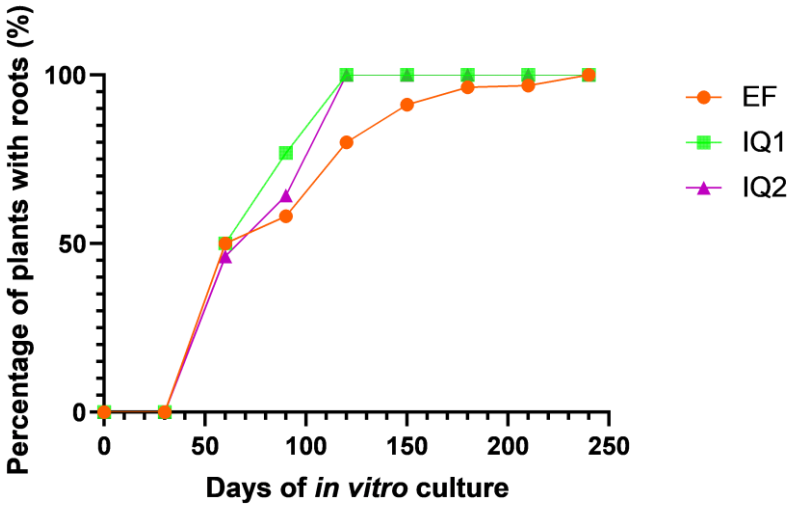


Figure 3. Percentage of rooting throughout 250 days of in vitro culture.

Abbreviation: EF, Experimental Farm; Iq1, Iñaquito 1; Iq2, Iñaquito 2. Kruskal-Wallis test (p=0.2319)

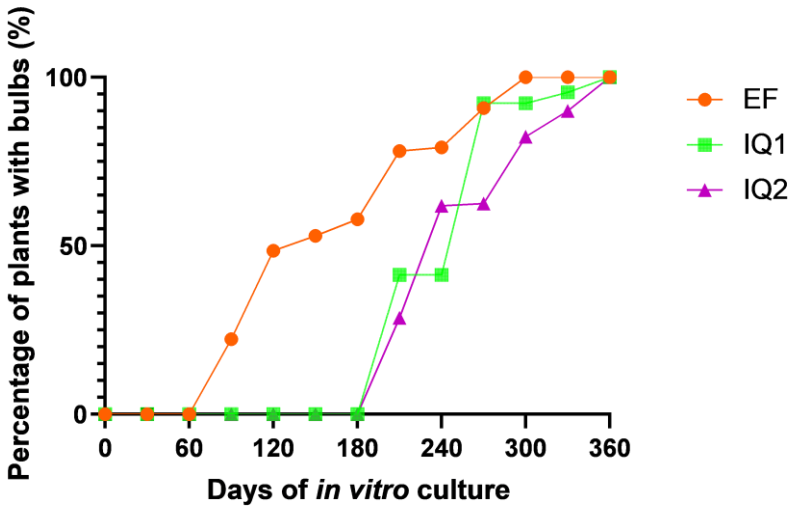


Figure 4. Percentage of bulbs formation throughout 360 days of *in vitro* culture.

Abbreviation: EF, Experimental Farm; Iq1, Iñaquito 1; Iq2, Iñaquito 2. Kruskal-Wallis test (p=0.0001)

DISCUSSION

Primer selection

The results of this study revealed that the virus detection protocol using RT-PCR and specific primers was effective. All five positive viral controls were amplified correctly with the selected primers. This could suggest that the literature-based primers and our designed primers performed efficiently. The primers successfully amplified the expected target region of the virus, enabling the detection of SLV and IYSV in shallot samples. The shallots analyzed did not have the following viruses: OYDV, ShVX, and LYSV. The OYDV has been detected before in shallot and garlic in the country (Heredia, 2016; Ramírez, 2012; Vélez et al., 2014), but LYSV and ShVX have only been detected in garlic (Granda et al., 2017; Oleas & Arahana, 2016).

Viral detection before *in vitro* culture

SLV detection

In the present study, SLV was present in 87,88% of the shallot plants analyzed. This virus belongs to the *Carlavirus* genus and *Betaflexiviridae* family. It is easily transmissible in sap and through aphids such as *Myzus ascalonicus* and possibly by *Aphis fabae* in a non-persistent manner (Pauzi et al., 2018). In previous studies carried out in Ecuador, SLV was detected in shallot (Ramirez, 2012) and garlic (*Allium sativum*) (Oleas & Arahana, 2016). In contrast to our study, in the study of Ramirez (2012), SLV was detected in 100% of shallots tested, while in garlic, the virus was presented in approximately 88.7% of the samples tested (Oleas & Arahana, 2016; Vélez et al., 2014). This virus is also present in other countries of South America, such as Argentina, Mexico, and Brazil (Mituti et al., 2011; Torrico et al., 2010; Velásquez-Valle et al., 2010), as well as other countries in Europe and Asia, such as Hungary, India, and China (Hu et al., 2015; Koczor et al., 2024; Majumder et al., 2008).

One study suggested that this virus is apparently omnipresent in shallots (Bos et al., 1978). Nevertheless, we found that 10.10% of samples do not have the virus. Other studies support our information, finding other viruses present in shallots and the absence of SLV. Heredia (2016) found that OYDV was present in 100% of the analyzed shallot samples, and no one had SLV. In another study, not all shallot samples had SLV, but other viruses such as ShVX, and new carlaviruses and potyviruses (Marais et al., 2019). That suggests that shallots may exhibit a diversity of viral infections and that SLV is not always found in shallot samples. Additionally, there is no available information on the evolution of SLV within the *Allium* genus.

IYSV detection

The second virus detected was IYSV. It was present in 29.29% of shallot samples. The samples collected from the market Iq2 had a higher percentage of IYSV. It is interesting that although Iq1 and Iq2 come from the same marketplace, 72% of Iq2, and only 16% of Iq2 had coinfection. This difference could be because the suppliers are different, and each of them manages the shallot crop in a different way. The growth and quality of shallot are influenced by genetic and environmental factors, water management, crop rotation, fertilization, pest and disease management (use of chemical pesticides) (Rovicky* et al., 2024; Sopha, 2020; Sutardi et al., 2022). This suggests that monitoring and controlling the viral diseases in crops is essential to maintain the quality of crops and virus-free plants.

IYSV belongs to the *Tospovirus* genus, *Bunyaviridae* family. IYSV is an emerging threat to onion bulb and seed production (Sharma & Cramer, 2023). In Ecuador, this virus has not been previously reported in shallots or garlic, but it has been reported in onions. One study found IYSV in twenty onion samples in Pichincha and Tungurahua provinces (Sivaprasad et al., 2016). Therefore, this is the first report of the presence of IYSV in shallots in the country. This virus has been reported to infect different *Allium* species including onion, shallot, leek,

garlic, and chive worldwide such as Netherlands, Germany, Austria, USA, Japan, South Africa, Mexico, Peru, Brazil, Uruguay, India, Japan, and Australia (Colnago et al., 2006; Cortês et al., 1998; Coutts et al., 2003; Creamer et al., 2004; Gawande et al., 2014; Gent et al., 2004; Leinhos et al., 2007; Mullis et al., 2006; Pozzer et al., 1999; Schwartz et al., 2002; Toit et al., 2007; Weilner & Bedlan, 2013). In the Mediterranean region, IYSV was reported in onion in Israel; shallot and onion in France; onion and leek in Spain, Slovenia, and Greece; onion, leek, and garlic in Egypt; onion in Italy and Tunisia (Córdoba-Sellés et al., 2005; Gent et al., 2004; Huchette et al., 2008; Katis et al., 2012).

The transmission of this virus is mainly due to vectors and plants that are the reservoirs of the virus (Bohdan et al., 2023). IYSV is transmitted by the vector *Thrips tabaci* Lindeman, which is also known as "onion trips" and transmits the virus in a persistent and propagative manner (Ávila-Alistac, 2024; Ramírez-Rojas et al., 2016). The detection of IYSV in shallot and onions in the country probably indicates the presence of the vector, which could need future research because this virus can affect other *Allium* species such as garlic and onion which are also grown in the country (Granda et al., 2017; Sivaprasad et al., 2016) and could cause future economic losses if the virus spreads.

In 2012, the IYSV disease had a 100% incidence on several hectares of onion in Tepalcingo (Mexico), one of the largest onion producers (Ramirez-Rojas et al., 2016). It also had a 100% incidence in Brazil, causing total production losses of seeds and onion bulbs (Ramírez-Rojas et al., 2016). IYSV is responsible for 10–15% of onion crop losses in the United States (Colnago et al., 2006). The infection makes the plant susceptible to other factors such as drought, high temperatures, excess irrigation, etc. (Katis et al., 2012; Ramírez-Rojas et al., 2016). The damage caused by IYSV occurs primarily because the viruses target chloroplasts and cause structural and functional changes in the infected cell. Some reports revealed that chlorosis caused by the virus correlates with chloroplast structure and membrane complex

malformation (Bhattacharyya & Chakraborty, 2018; Zhao et al., 2019). In one study, chloroplasts of IYSV-infected onions were observed to undergo visible malformations, including a broken chloroplast envelope, thin and irregular outer membranes, disorganization of the stromal lamella and grana, and the presence of spherical IYSV-like particles accumulated in the chloroplast that resembled inclusion bodies (Abdelkhalek et al., 2019).

The symptoms produced by IYSV are diamond-shaped lesions of 1–5 cm and limited by a chlorotic zone. This symptomatology was evident on some leaves of the shallot plants collected from OM, Iq1, and Iq2. The necrotic areas can be colonized by some secondary pathogens, such as *Alternaria spp*. and *Stemphylum spp*., making a difficult diagnosis (Colnago et al., 2006). Therefore, this detection could help implement control and prevention methods to stop the virus from spreading and causing significant economic and crop losses in the future. In addition, accurate identification of the vector and study of its biology could provide valuable information that can help growers control the disease.

Coinfection

Coinfections are frequent in shallot plants (Marais et al., 2019), and in this study, 27.27% of shallots were co-infected with both viruses (SLV and IYSV). This coinfection was only visible in OM, Iq1, and Iq2. Infections of IYSV with *Carlavirus* and *Allexivirus* are possible, and when it happens, there is a synergistic effect on symptomatology that produces huge yield losses (Shevchenko et al., 2024). It is probable that this problem is being faced by farmers in the countryside. We suggest that this is one of the reasons that influence the country's inability to meet the demand for shallots and the need to import. There is a lack of information that helps them to control the disease because, in Ecuador, virus monitoring in shallots and other crops of the *Allium* genus is not as widely documented as in other countries. There are only research and programs related to crop health and the prevention of viral diseases,

especially by universities such as ESPAM and USFQ (Heredia, 2016; Ramírez, 2012; Vega et al., 2015; Vélez et al., 2014).

In vitro culture

First phase: Disinfection of plant material

The results showed that our developed standardized disinfection protocol maintains the sterility of shallot basal discs in 35% to 44.4% of all trials. These percentages are not as high as expected but allowed us to obtain enough sterile plant material to continue with the trials. The most common pests that attack shallots are fungi, especially *Fusarium oxysporum*, *Colletotrichum gloeosporioides, Alternaria porii,* and *Pythopthora porii*; each of them produces a different disease in the plant (Muhammad et al., 2022). The fungicide that we used as part of the disinfection process and that influenced this sterility percentage was Mertect, which contains Thiabendazole and is considered highly effective with a broad spectrum of activity against several types of pathogenic fungi (Papadopoulou et al., 2018). A previous study by Ramírez (2012) obtained 100% sterility. In this study, the fungicide Benomyl was used. This fungicide is well known for its efficiency in inhibiting most taxonomic groups of fungi (Coronel et al., 2022; Summerbell, 1993). However, it is teratogenic and toxic (Kara et al., 2020), and is banned in Ecuador (Kara et al., 2020).

All trials achieved 100% basal disc sprouting. This is an important finding because, unlike other studies, we do not use a drying or cooling period (Ramírez, 2012; Vega et al., 2015). This approach is beneficial since these processes typically take about 60 days to complete. Some research indicates that the sprouting of shallot bulbs occurs after a certain duration following harvest, as they go through a dormancy period (Bufler, 2009). However, our results showed that all discs sprouted only after 15 days of culture.

Second phase: Shoot tip culture

In this study, we use shoot tip culture to eradicate viruses infecting shallots. In *vitro culture*, especially meristem and shoot tip culture, has been used for virus-free plant propagation because of its efficacy. Meristem culture was one of the main methods used for SLV and OYDV eradication (Wang et al., 2021). This shoot tip culture allowed more than 57% of shoot tips to develop in plantlets, 100% of shallots to develop roots, and more than 82% of plantlets to develop bulbs up to 300 days of culture. Our regeneration results are higher than those obtained by Ramírez (2012) and Vega et al. (2015) of 30% to 40% and 68%, respectively. In this study, we observed that the addition of ancymidol (an inhibitor of gibberellin biosynthesis) to the M3 medium promoted the percentage of bulb formation. Ancymidol stimulates bulbing and rhizogenesis because it causes a decrease in the sucrose content of leaf bases and increases the contents of glucose, fructan, and fructose under these conditions (Saos, 2002). The bulb formation percentage is higher compared with the 70% obtained by Vega et al. (2015).

There was a significant difference in time to bulb formation in each trial depending on the source of shallots analyzed. The shoots of the plants coming from EF grew into plantlets and developed bulbs after 90 days, while for Iq1 and Iq2, they started later at 210 days of culture. We suggest that these differences could be due to the initial plant material quality due to the source of shallots being the only variant among the released trials. Furthermore, shallots that belonged to EF were purple, and those of Iq1 and Iq2 were white. Although white and purple shallot bulbs do not differ as much in chloroplast and mitochondrion DNA, karyotype, anther, or leaf morphology (Perković et al., 2020), differences in biochemical and morphological profiles have been observed depending on the accession (Major et al., 2018). These differences influence their time of bulbing and rhizogenesis.

All plants with root and bulb development were successfully acclimatized. This represents a significant achievement because the previous studies did not obtain any acclimatized plants (Ramírez, 2012) or only 50% of plants acclimatized (Vega et al., 2015). It is essential to continue studies that aid in better understanding the factors affecting shallot development, particularly bulb formation, and to produce and select the highest quality plants for *in vitro* propagation. Shoot tip culture and tissue culture-derived seedlings are considered a new source to improve and develop *in vitro* shallot research (Marlin et al., 2021).

Viral detection after *in vitro* culture

In our study, SLV remained present in 100% of all shallots, while IYSV was eliminated after shoot tip culture. In this case, there was a simultaneous infection of a *Carlavirus* with a *Tospovirus*. Some studies indicate that coinfections are more challenging to eliminate than infections caused by a single virus (Szabó et al., 2024), an aspect that we could see in our results. The differences in virus eradication frequencies can be attributed to the specific virus-host combinations (Wang et al., 2021).

SLV

SLV is asymptomatic and is not considered to have a significant economic impact on shallots unless other viruses, the problem is when there are simultaneous infections (Katis et al., 2012; Marais et al., 2019). The shoot tip size is critical for virus eradication (Wang et al., 2018), and this could be one of the reasons that may explain why the virus has not been eliminated. Shoot tips have the meristem but also a portion of differentiated tissue, and it has been shown that viruses tend to migrate through the vascular elements (Hipper et al., 2013). Most studies analyzing explant size in meristem culture have been conducted on garlic and few on shallot plants. Using meristem culture (0.5 to 0.8 mm), Walkey et al. (1987) obtained 25% garlic plants and 26% shallot plants free of viruses such as SLV and OYDV. In the study of

garlic, Verbeek et al. (1995) obtained 91 to 100% OYDV-G-free plants with a meristem of 0.15 to 0.3 mm, while a 0.2 mm meristem culture produced 62-65% of OYDV, LYSV, and GCLV-free plants. This method also efficiently eliminated *Allexivirus* (Shiboleth et al., 2001). In the study of shallots by Wang et al. (2021), the 0.5 mm meristem culture resulted in 10% OYDV and 15% SLV-free plants (Wang et al., 2021). On the other hand, shoot tip culture was used and shown to produce plants with an elimination rate of virus of 96.2% for SLV and OYDV in the third generation of plants (Lou et al., 2022).

Comparing the viral eradication efficiency in garlic of shoot tip (1–1.5 cm) with meristem (0.1–0.3 mm) cultures in OYDV elimination resulted in 26% and 40% virus-free plants, respectively (Vieira et al., 2015). Meristem culture typically results in a higher number of virus-free plants; however, a significant challenge lies in the process of meristem cutting. Smaller meristem explants stand a better chance of being virus-free because more differentiated tissue surrounding the meristem provides a greater risk of virus accumulation (Panattoni et al., 2013; Kahane et al., 1992). However, the survival and regeneration rates are lower in meristems than in shoot tips due to this differentiated tissue increases the regeneration probability (Maliogka et al., 2015). We could suggest using a smaller shoot tip in future studies to increase the elimination rate of SLV. However, as shown in the studies mentioned above, SLV is a complex virus that is difficult to eliminate at 100% with a single treatment. One hypothesis suggests that the antiviral barriers of the meristem may be active all the time and some viruses have developed the ability to invade the meristem; or that these barriers are activated only in the presence of viruses, and some viral species that do not activate them, can invade the meristem (Bradamante et al., 2021). This argument is supported by a recent study on SLV virus localization within the meristem that revealed that only 20% to 27% of the 0.5 mm shallot meristematic region was SLV-free (Magyar-Tábori et al., 2021), implying that shoot tip culture

needs a combination with other treatments such as chemotherapy to completely eliminate this virus.

This is our preliminary screening before testing other methods or combined methods. In future analysis, we recommend using combination treatments to get SLV-free shallot plants. In a study, the combination of thermotherapy, chemotherapy, and meristem culture led to an SLV elimination in 100% of garlic plants (Benke et al., 2023). Another report showed that the combination of thermotherapy with meristem culture eliminated 80% of SLV in shallot shoots (Wang et al., 2021). However, SLV is not a virus that causes great problems, so the risk-benefit of using combined treatments should be evaluated, as it has been seen that they can cause negative effects on the plant growth (Magyar-Tábori et al., 2021). Thus, it is essential to conduct additional studies to evaluate each mentioned method with shoot tip culture to determine the best combination methods to achieve a high regeneration rate and virus-free shallots. This is crucial for achieving the goal of obtaining and propagating virus-free plants.

In our study, four shallot samples from assays 2 and 3 were negative for SLV before *in vitro* culture and positive after shoot tip culture. These results were not as expected. In the literature, it is reported that the amount of SLV varies according to the plant stage, and it is higher on adventitious shoots and 14 DAP (diameter at breast height) leaves (Pauzi et al., 2018). This could suggest that the leaves of shallot samples taken before *in vitro* culture probably contained the virus; however, the concentration was too low to enable the detection by conventional PCR. Another study also showed that the concentration of certain viruses tends to increase after an ineffective eradication treatment (Velásquez-Valle et al., 2016). It could suggest that the virus increased its amount. SLV is a latent virus, and during viral latency, viral replication is not present due to viruses remaining in a dormant state. Nevertheless, a latent virus can reactivate and replicate under certain conditions, such as environmental stress

(produced by the *in vitro* culture process itself) or during specific developmental stages of the host plant (De Klerk, 2007; Takahashi et al., 2019).

IYSV

The shoot tip culture effectively eliminated IYSV, resulting in 100% virus-free plants, regardless of the source (Iq1, Iq2). Since 1999, IYSV has been included in the European and Mediterranean Plant Protection Organization (EPPO) alert list due to its potential hazard to onion crops (Cordoba et al., 2005). It is an industry concern due to the limited availability of effective treatment options to control the virus and its vector (Tripathi et al., 2015). Unfortunately, until 2023, no chemical and biological methods were available to eliminate the virus within the plant (Sharma & Cramer, 2023). Our study demonstrated the effectiveness of shoot tip culture, revealing its potential for future applications. Shoot tip culture should be applied to eliminate other symptomatic viruses that reduce crop quality, such as ShVX, LYSV, OYDV, etc., as this method allows a high rate of regeneration and viral elimination. It would be interesting to know if it is possible to eliminate other problematic and symptomatic viruses in plants.

CONCLUSION

This study reports an efficient protocol for the molecular detection of two of the most common viruses in shallot samples, SLV and IYSV. This is the first report of the presence of IYSV in shallots in Ecuador. This detection could help implement control, monitoring, and prevention methods to stop the virus from spreading and causing significant economic and crop losses in the future in shallot or other *Allium* crops. The presence of the virus implies the presence of its vector, and the accurate identification of the vector and study of its biology could provide valuable information about IYSV that can help growers to control the disease. The results of our shoot tip culture proved to be effective in eliminating IYSV, which in other countries has caused significant economic losses by impacting crop quality and yield and no chemical and biological methods were available to eliminate the virus. Although our study focused on the detection of five viruses, other viruses may be present in shallots, so it would be interesting in further studies to use third-generation sequencing to understand the complete shallot virome. The *in vitro* culture was efficient and allowed a high percentage of plantlet development, rooting, bulb formation, and acclimatization. Therefore, shoot tip culture could be a valuable tool for future research and an effective method to eradicate this symptomatic virus in shallots. Good quality plants (free of IYSV and effectively acclimatized) that were obtained in this study were given to local farmers, thus allowing them to have good plant material for propagation in the field. Research of this type has a scientific contribution that also implies an input for improved agriculture.

REFERENCES

Abdelkhalek, A., Qari, S. H., & Hafez, E. (2019). Iris yellow spot virus–induced chloroplast malformation results in male sterility. *Journal of Biosciences*, *44*(6), 142. https://doi.org/10.1007/s12038-019-9960-9

Ávila-Alistac, N. (2024). Patosistema de Iris yellow spot orthotospovirus, hospederos del virus y el vector (Thrips tabaci). . . *Vol.*, *42*.

Benke, A. P., Krishna, R., Khandagale, K., Gawande, S., Shelke, P., Dukare, S., Dhumal, S., Singh, M., & Mahajan, V. (2023). Efficient Elimination of Viruses from Garlic Using a Combination of Shoot Meristem Culture, Thermotherapy, and Chemical Treatment. *Pathogens*, *12*(1), 129. https://doi.org/10.3390/pathogens12010129

Bhattacharyya, D., & Chakraborty, S. (2018). Chloroplast: The Trojan horse in plant–virus interaction. *Molecular Plant Pathology*, *19*(2), 504–518. https://doi.org/10.1111/mpp.12533

Bohdan, M. M., Kyrychenko, A. M., Shcherbatenko, I. S., & Kraeva, H. V. (2023). Weed Plants of the Asteraceae and Malvaceae Families as Reservoirs of Harmful Viruses of Vegetable Crops in Ukraine and the World. *Mikrobiolohichnyi Zhurnal*, *85*(5), 66–76. https://doi.org/10.15407/microbiolj85.05.066

Colnago, P., Achigar, R., Ravelino, P. G., Peluffo, S., Idiarte, H. G., Pianzzola, M. J., & Galván, G. A. (2006). *PRESENCIA DEL VIRUS IRIS YELLOW SPOT VIRUS (IYSV) EN SEMILLEROS DE CEBOLLA*.

Cordoba, C., Priego, L., Gómez, R. M., Jordá, C., & Tobarra, M. (2005). Iris yellow spot virus (IYSV): Nuevo virus en el cultivo de la cebolla en España. *Boletín de Sanidad Vegetal. Plagas, ISSN 0213-6910, Vol. 31, Nº 3, 2005, Pags. 425-430*.

Córdoba-Sellés, C., Martínez-Priego, L., Muńoz-Gómez, R., & Jordá-Gutiérrez, C. (2005). *Iris yellow spot virus: A New Onion Disease in Spain. 89*(11), 1243.

Coronel, A. C., Parraguirre Lezama, C., Pacheco Hernández, Y., Santiago Trinidad, O., Rivera Tapia, A., & Romero-Arenas, O. (2022). Efficacy of Four In Vitro Fungicides for Control of Wilting of Strawberry Crops in Puebla-Mexico. *Applied Sciences*, *12*(7), 3213. https://doi.org/10.3390/app12073213

Corrado, C. L., Micali, G., Mauceri, A., Bertin, S., Sunseri, F., Abenavoli, M. R., & Tiberini, A. (2024). Study on Italian Onion Cultivars/Ecotypes towards Onion Yellow Dwarf Virus (OYDV) Infection. *Horticulturae*, *10*(1), 73. https://doi.org/10.3390/horticulturae10010073

Cortês, I., Livieratos, I. C., Derks, A., Peters, D., & Kormelink, R. (1998). Molecular and Serological Characterization of Iris Yellow Spot Virus, a New and Distinct Tospovirus Species. *Phytopathology®*, *88*(12), 1276–1282. https://doi.org/10.1094/PHYTO.1998.88.12.1276

Coutts, B. A., McMichael, L. A., Tesoriero, L., Rodoni, B. C., Wilson, C. R., Wilson, A. J., Persley, D. M., & Jones, R. A. C. (2003). Iris yellow spot virus found infecting onions in three Australian states. *Australasian Plant Pathology*, *32*(4), 555. https://doi.org/10.1071/AP03060

Creamer, R., Sanogo, S., Moya, A., Romero, J., Molina-Bravo, R., & Cramer, C. (2004). *Iris yellow spot virus* on Onion in New Mexico. *Plant Disease*, *88*(9), 1049–1049. https://doi.org/10.1094/PDIS.2004.88.9.1049C

FAOSTAT. (2024). *Organización para la agricultura y la alimentación de las naciones unidas.* https://www.fao.org/faostat/es/#data/QCL

Fritsch, R. M., & Friesen, N. (2002). Evolution, domestication and taxonomy. In H. D. Rabinowitch & L. Currah (Eds.), *Allium crop science: Recent advances* (1st ed., pp. 5–30). CABI Publishing. https://doi.org/10.1079/9780851995106.0005

Gargi, B., Singh, P., Painuli, S., Rai, N., Semwal, P., Cruz-Martins, N., & Sharma, R. (2024). Literature-based screening and bibliometric analysis of the chemical composition, antioxidant and antimicrobial potential of essential oils isolated from Allium genus: 23 years of investigation. *Pharmacological Research - Modern Chinese Medicine*, *10*, 100354. https://doi.org/10.1016/j.prmcm.2023.100354

Gawande, S., Gurav, V., Ingle, A., & Gopal, J. (2014). First Report of Iris yellow spot virus Infecting Allium tuberosum in India. *Plant Disease*, *98*, 1161. https://doi.org/10.1094/PDIS-01-14-0002-PDN

Gent, D. H., Schwartz, H. F., & Khosla, R. (2004). Distribution and Incidence of *Iris yellow spot virus* in Colorado and Its Relation to Onion Plant Population and Yield. *Plant Disease*, *88*(5), 446–452. https://doi.org/10.1094/PDIS.2004.88.5.446

Granda, R., Landázuri, G., & Arkhipov, A. V. (2017). First Report of *Shallot virus X* in Garlic in Ecuador. *Plant Disease*, *101*(6), 1066. https://doi.org/10.1094/PDIS-11-16-1558-PDN

Gunaeni, N., Adiyoga, W., Rosliani, R., & Sulastrini, I. (2021). The Effect of Plant Growth Regulators and Planting Density against Viral Infection and the Production from Bulbs of True Shallot Seed in the Highlands. *IOP Conference Series: Earth and Environmental Science*, *752*(1), 012033. https://doi.org/10.1088/1755-1315/752/1/012033

Heredia, S. (2016). *Erradicación de Onion Yellow Dwarf Virus (OYDV) en cebolla Shallot (Allium cepa var. Aggregatum), mediante el Cultivo de meristemas, quimioterapia y termoterapia para la producción de bulbos libres de virus.*

Hipper, C., Brault, V., Ziegler-Graff, V., & Revers, F. (2013). Viral and Cellular Factors Involved in Phloem Transport of Plant Viruses. *Frontiers in Plant Science*, *4*. https://doi.org/10.3389/fpls.2013.00154

Hu, X., Lei, Y., Wang, P., Tang, L., He, C., Song, Y., Xiong, X., & Nie, X. (2015). Development of a multiplex reverse transcription-PCR assay for simultaneous detection of garlic viruses. *Journal of Integrative Agriculture*, *14*(5), 900–908. https://doi.org/10.1016/S2095-3119(14)60892-3

Huchette, O., Bellamy, C., Filomenko, R., Pouleau, B., Seddas, S., & Pappu, H. R. (2008). *Iris yellow spot virus* on Shallot and Onion in France. *Plant Health Progress*, *9*(1), 46. https://doi.org/10.1094/PHP-2008-0610-01-BR

Hull, R. (2014). Ecology, Epidemiology, and Control of Plant Viruses. In *Plant Virology* (pp. 809–876). Elsevier. https://doi.org/10.1016/B978-0-12-384871-0.00014-5

Jaramillo, G. (2020). *Shallot virus X: a hardly known pathogen of the genus Allium*.

Kara, M., Oztas, E., Ramazanoğulları, R., Kouretas, D., Nepka, C., Tsatsakis, A. M., & Veskoukis, A. S. (2020). Benomyl, a benzimidazole fungicide, induces oxidative stress and apoptosis in neural cells. *Toxicology Reports*, *7*, 501–509. https://doi.org/10.1016/j.toxrep.2020.04.001

Karjadi, A. K., Aswani, N., & Gunaeni, N. (2023). Elimination of systemic viral disease in shallot (Allium ascolonicum L) var. Bima Brebes through unconventional approach. *IOP Conference Series: Earth and Environmental Science*, *1230*(1), 012099. https://doi.org/10.1088/1755-1315/1230/1/012099

Katis, N. I., Maliogka, V. I., & Dovas, C. I. (2012). Viruses of the Genus Allium in the Mediterranean Region. In *Advances in Virus Research* (Vol. 84, pp. 163–208). Elsevier. https://doi.org/10.1016/B978-0-12-394314-9.00005-1

Kawakubo, S., Kim, H., Takeshita, M., & Masuta, C. (2023). Host-specific adaptation drove the coevolution of leek yellow stripe virus and *Allium* plants. *Microbiology Spectrum*, *11*(5), e02340-23. https://doi.org/10.1128/spectrum.02340-23

Kim, H., Kawakubo, S., Takahashi, H., & Masuta, C. (2023). Two mutually exclusive evolutionary scenarios for allexiviruses that overcome host RNA silencing and autophagy by regulating viral CRP expression. *PLOS Pathogens*, *19*(6), e1011457. https://doi.org/10.1371/journal.ppat.1011457

Koczor, Á., Ádám, J., Ágoston, J., Salánki, K., & Palkovics, L. (2024). Investigation of viral diseases of garlic (Allium sativum L.), new primers for RT-PCR detection and diversity of garlic viruses in Hungary. *Physiological and Molecular Plant Pathology*, *134*, 102394. https://doi.org/10.1016/j.pmpp.2024.102394

Krishna, R., Ansari, W. A., Khandagale, K., Benke, A. P., Soumia, P. S., Manjunathagowda, D. C., Gawande, S. J., Ade, A. B., Mokat, D. N., & Singh, M. (2022). Chapter 14—Meristem culture: A potential technique for in vitro virus-free plants production in vegetatively propagated crops. In A. Chandra Rai, A. Kumar, A. Modi, & M. Singh (Eds.), *Advances in Plant Tissue Culture* (pp. 325–343). Academic Press. https://doi.org/10.1016/B978-0-323-90795-8.00017-5

Leinhos, G., Müller, J., Heupel, M., & Krauthausen, H.-J. (2007). *Iris Yellow Spot Virus an Bund- und Speisezwiebeln – erster Nachweis in Deutschland*.

Leino, M. W., Solberg, S. Ø., Tunset, H. M., Fogelholm, J., Strese, E.-M. K., & Hagenblad, J. (2018). Patterns of Exchange of Multiplying Onion (Allium cepa L. Aggregatum-Group) in Fennoscandian Home Gardens. *Economic Botany*, *72*(3), 346–356. https://doi.org/10.1007/s12231-018-9426-2

Lima, J. A., Q. Nascimento, A. K., Radaelli, P., & E., D. (2012). Serology Applied to Plant Virology. In M. Al-Moslih (Ed.), *Serological Diagnosis of Certain Human, Animal and Plant Diseases*. InTech. https://doi.org/10.5772/38038

López-Moya, J. J., & García, J. A. (2008). Potyviruses. In B. W. J. Mahy & M. H. V. Van Regenmortel (Eds.), *Encyclopedia of Virology (Third Edition)* (pp. 313–322). Academic Press. https://doi.org/10.1016/B978-012374410-4.00475-1

Lou, H., Liu, Z., & Xu, Q. (2022). Detection and elimination of shallot latent virus and onion yellow dwarf virus for potato onion (Allium cepa L. var. Aggregatum Don). *Annals of Applied Biology*, *182*. https://doi.org/10.1111/aab.12801

Magyar-Tábori, K., Mendler-Drienyovszki, N., Hanász, A., Zsombik, L., & Dobránszki, J. (2021). Phytotoxicity and Other Adverse Effects on the In Vitro Shoot Cultures Caused by Virus Elimination Treatments: Reasons and Solutions. *Plants*, *10*(4), 670. https://doi.org/10.3390/plants10040670

Mahmoud, S., A. Abo-El, S., M. El-Boro, A., & H. Abdel-G, M. (2007). Identification of Onion yellow dwarf potyvirus as One of the Major Viruses Infecting Garlic in Egypt. *International Journal of Virology*, *4*(1), 1–13. https://doi.org/10.3923/ijv.2008.1.13

Major, N., Goreta Ban, S., Urlić, B., Ban, D., Dumičić, G., & Perković, J. (2018). Morphological and Biochemical Diversity of Shallot Landraces Preserved Along the Croatian Coast. *Frontiers in Plant Science*, *9*, 1749. https://doi.org/10.3389/fpls.2018.01749

Majumder, S., Baranwal, V. K., & Joshi, S. (2008). Simultaneous detection of onion yellow dwarf virus and shallot latent virus in infected leaves and cloves of garlic by duplex RT-PCR. *Journal of Plant Pathology*, *90*(2), 371–374. Scopus.

Maliogka, V. I., Martelli, G. P., Fuchs, M., & Katis, N. I. (2015). Control of Viruses Infecting Grapevine. In *Advances in Virus Research* (Vol. 91, pp. 175–227). Elsevier. https://doi.org/10.1016/bs.aivir.2014.11.002

Marais, A., Faure, C., Theil, S., & Candresse, T. (2019). Characterization of the virome of shallots affected by the shallot mild yellow stripe disease in France. *PLOS ONE*, *14*(7), e0219024. https://doi.org/10.1371/journal.pone.0219024

Marlin, M., Handajani, M., Yulian, Y., Rustikawati, R., & Herawati, R. (2021). *Induction of Plantlet Regeneration on Shallot (* Allium cepa *var.* Aggregatum *):* International Seminar on Promoting Local Resources for Sustainable Agriculture and Development (ISPLRSAD 2020), Bengkulu, Sumatra, Indonesia, Indonesia. https://doi.org/10.2991/absr.k.210609.038

Matthews, R. E. F. (1967). 5—Serological Techniques for Plant Viruses. In K. MARAMOROSCH & H. KOPROWSKI (Eds.), *Methods in Virology* (Vol. 3, pp. 199–241). Elsevier. https://doi.org/10.1016/B978-1-4832-3219-5.50011-1

Mituti, T., Marubayashi, J. M., Moura, M. F., Krause-Sakate, R., & Pavan, M. A. (2011). First Report of *Shallot latent virus* in Garlic in Brazil. *Plant Disease*, *95*(2), 227–227. https://doi.org/10.1094/PDIS-08-10-0599

Moldovan, C., Frumuzachi, O., Babotă, M., Barros, L., Mocan, A., Carradori, S., & Crişan, G. (2022). Therapeutic Uses and Pharmacological Properties of Shallot (Allium ascalonicum): A Systematic Review. *Frontiers in Nutrition*, *9*, 903686. https://doi.org/10.3389/fnut.2022.903686

Mullis, S. W., Gitaitis, R. D., Nischwitz, C., Csinos, A. S., Rafael Mallaupoma, Z. C., & Inguil Rojas, E. H. (2006). First Report of Onion (*Allium cepa*) Naturally Infected with *Iris yellow spot virus* in Peru. *Plant Disease*, *90*(3), 377–377. https://doi.org/10.1094/PD-90-0377A

NCBI. (2024). https://www.ncbi.nlm.nih.gov/tools/primer-blast/

Nehra, N. S., & Kartha, K. K. (1994). Meristem and Shoot Tip Culture: Requirements and Applications. In I. K. Vasil & T. A. Thorpe (Eds.), *Plant Cell and Tissue Culture* (pp. 37–70). Springer Netherlands. https://doi.org/10.1007/978-94-017-2681-8_3

Nhung, T., & Quoc, L. (2024). Efficacy of Black Shallot Extract in Analgesic and Antipyretic Activities in Experimental Mice. *Tropical Journal of Natural Product Research, 8*(3). https://doi.org/10.26538/tjnpr/v8i3.20

Nie, X., & Singh, R. P. (2017). Viroid Detection and Identification by Bioassay. In *Viroids and Satellites* (pp. 347–356). Elsevier. https://doi.org/10.1016/B978-0-12-801498-1.00033-4

Oleas, A., & Arahana, V. (2016). First report of leek yellow stripe virus, shallot latent virus, and onion yellow dwarf virus in Garlic from Ecuador. *Plant Disease, 100*(1), 232. Scopus. https://doi.org/10.1094/PDIS-03-15-0318-PDN

Pangestuti, Prahardini, P. E. R., Rosliani, R., Rahayu, M., Tandry, O., Saidah, & Pramono, J. (2023). Seedling production management of Shallots from seeds based on local wisdom technology. *IOP Conference Series: Earth and Environmental Science*, *1230*(1), 012213. https://doi.org/10.1088/1755-1315/1230/1/012213

Papadopoulou, E. S., Genitsaris, S., Omirou, M., Perruchon, C., Stamatopoulou, A., Ioannides, I., & Karpouzas, D. G. (2018). Bioaugmentation of thiabendazole-contaminated soils from a wastewater disposal site: Factors driving the efficacy of this strategy and the diversity of the indigenous soil bacterial community. *Environmental Pollution*, *233*, 16–25. https://doi.org/10.1016/j.envpol.2017.10.021

Pareek, S., Sagar, N. A., Sharma, S., & Kumar, V. (2017). Onion (*Allium cepa* L .). In E. M. Yahia (Ed.), *Fruit and Vegetable Phytochemicals* (1st ed., pp. 1145–1162). Wiley. https://doi.org/10.1002/9781119158042.ch58

Pauzi, Y. S., Lestari, S. M., & Hidayat, S. H. (2018). Variations of *Garlic Common Latent Virus* and *Shallot Latent Virus Concentration* on Shallot and Garlic. *IOP Conference Series: Earth and Environmental Science*, *197*, 012045. https://doi.org/10.1088/1755-1315/197/1/012045

Pozzer, L., Bezerra, I. C., Kormelink, R., Prins, M., Peters, D., Resende, R. D. O., & De Ávila, A. C. (1999). Characterization of a Tospovirus Isolate of Iris Yellow Spot Virus Associated with a Disease in Onion Fields in Brazil. *Plant Disease*, *83*(4), 345–350. https://doi.org/10.1094/PDIS.1999.83.4.345

Purnamasari, N. R., Haryati, Y., Hamdani, K. K., & Dianawati, M. (2023). Effect of pollination method on true shallot seed formation improvement. *IOP Conference Series: Earth and Environmental Science*, *1230*(1), 012123. https://doi.org/10.1088/1755-1315/1230/1/012123

Qu, W., & Zhang, C. (2015). Selecting Specific PCR Primers with MFEprimer. In C. Basu (Ed.), *PCR Primer Design* (Vol. 1275, pp. 201–213). Springer New York. https://doi.org/10.1007/978-1-4939-2365-6_15

Rabinowitch, H. D., & Kamenetsky, R. (2002). Shallot (*Allium cepa* , Aggregatum group). In H. D. Rabinowitch & L. Currah (Eds.), *Allium crop science: Recent advances* (1st ed., pp. 409–430). CABI Publishing. https://doi.org/10.1079/9780851995106.0409

Rajan, P., Lada, R. R., & MacDonald, M. T. (2019). Advancement in Indoor Vertical Farming for Microgreen Production. *American Journal of Plant Sciences*, *10*(08), 1397–1408. https://doi.org/10.4236/ajps.2019.108100

Ramírez, D. (2012). *Regeneración de plántulas de chalote (Allium cepa var. Aggregatum) libres de los virus Latente del Chalote y del Enanismo Amarillo de la Cebolla por medio de cultivo de meristema apical y quimioterapia*. Universidad San Francisco de Quito.

Ramírez-Rojas, S., Ornelas-Ocampo, K., Osuna-Canizalez, F. D. J., Bartolo-Reyes, J. C., Varela-Loza, V., Hernández-Romano, J., & Ochoa-Martínez, D. L. (2016). Detección de Iris yellow spot virus en cebolla cultivada en Tepalcingo, Morelos, México. *Revista Mexicana de Fitopatología, Mexican Journal of Phytopathology*, *34*(3). https://doi.org/10.18781/R.MEX.FIT.1604-1

Rio, D. C., Ares, M., Hannon, G. J., & Nilsen, T. W. (2010). Purification of RNA Using TRIzol (TRI Reagent). *Cold Spring Harbor Protocols*, *2010*(6), pdb.prot5439. https://doi.org/10.1101/pdb.prot5439

Rovicky*, A., Widowati, W., & Astutik, A. (2024). Pest and Disease Control Strategies to Increase the Productivity of Shallot Plants (Allium ascalonium L.). *Riwayat: Educational Journal of History and Humanities*, *7*(3), 1253–1260. https://doi.org/10.24815/jr.v7i3.40246

Saos, F. L. G.-L. (2002). In vitro Bulb Development in Shallot (Allium cepa L. Aggregatum Group): Effects of Anti-gibberellins, Sucrose and Light. *Annals of Botany*, *89*(4), 419–425. https://doi.org/10.1093/aob/mcf063

Schwartz, H. F., Brown, W. M., Blunt, T., & Gent, D. H. (2002). *Iris yellow spot virus* on Onion in Colorado. *Plant Disease*, *86*(5), 560–560. https://doi.org/10.1094/PDIS.2002.86.5.560D

Sharma, S., & Cramer, C. S. (2023). Reduced Iris yellow spot symptom expression in the selected onion germplasm. *Vegetable Research*, *3*(1), 0–0. https://doi.org/10.48130/VR-2023-0026

Shen, W., Shi, Y., Dai, Z., & Wang, A. (2020). The RNA-Dependent RNA Polymerase NIb of Potyviruses Plays Multifunctional, Contrasting Roles during Viral Infection. *Viruses*, *12*(1), 77. https://doi.org/10.3390/v12010077

Shevchenko, O., Snihur, H., Shevchenko, T., & Budzanivska, I. (2024). Onion and Garlic. In *Viral Diseases of Field and Horticultural Crops* (pp. 505–512). Elsevier. https://doi.org/10.1016/B978-0-323-90899-3.00048-3

Shiboleth, Y. M., Gal-On, A., Koch, M., Rabinowitch, H. D., & Salomon, R. (2001). Molecular characterisation of Onion yellow dwarf virus (OYDV) infecting garlic (Allium sativum L.) in Israel: Thermotherapy inhibits virus elimination by meristem tip culture. *Annals of Applied Biology*, *138*(2), 187–195. https://doi.org/10.1111/j.1744-7348.2001.tb00101.x

Sivaprasad, Y., Garrido, P., Mendez, K., Garrido, A., & Ramos, L. (2016). First report of *Iris yellow spot virus* infecting onion in the Pichincha and Tungurahua provinces of Ecuador. *New Disease Reports*, *33*(1), 16–16. https://doi.org/10.5197/j.2044-0588.2016.033.016

Sopha, G. A. (2020). INFLUENCE OF PLANT DENSITY, COMPOST AND BIOFERTILIZER ON TRUE SHALLOT SEED GROWTH IN ALLUVIAL SOIL. *Indonesian Journal of Agricultural Science*, *21*(2), 70. https://doi.org/10.21082/ijas.v21n2.2020.p70-77

Summerbell, R. C. (1993). The benomyl test as a fundamental diagnostic method for medical mycology. *Journal of Clinical Microbiology*, *31*(3), 572–577. https://doi.org/10.1128/jcm.31.3.572-577.1993

Sutardi, Kristamtini, Purwaningsih, H., Widyayanti, S., Arianti, F. D., Pertiwi, M. D., Triastono, J., Praptana, R. H., Malik, A., Cempaka, I. G., Yusuf, Yufdy, M. P., Anda, M., & Wihardjaka, A. (2022). Nutrient Management of Shallot Farming in Sandy Loam Soil in Tegalrejo, Gunungkidul, Indonesia. *Sustainability*, *14*(19), 11862. https://doi.org/10.3390/su141911862

Toit, L. J. du, Burger, J. T., McLeod, A., Engelbrecht, M., & Viljoen, A. (2007). Iris yellow spot virus in Onion Seed Crops in South Africa. *Plant Disease, 91*(9), 1203–1203. https://doi.org/10.1094/PDIS-91-9-1203A

Torres, J. (2014). *Evaluación agronómica de diez "familias" seleccionadas de Chalote (Allium cepa variedad aggregatum) en Puembo-Pichincha*.

Torrico, A. K., Cafrune, E. E., & Conci, V. C. (2010). First Report of *Shallot latent virus* in Garlic in Argentina. *Plant Disease, 94*(7), 915–915. https://doi.org/10.1094/PDIS-94-7-0915B

Tripathi, D., Raikhy, G., Goodin, M. M., Dietzgen, R. G., & Pappu, H. R. (2015). In Vivo Localization of Iris yellow spot Tospovirus (Bunyaviridae)-Encoded Proteins and Identification of Interacting Regions of Nucleocapsid and Movement Proteins. *PLOS ONE*, *10*(3), e0118973. https://doi.org/10.1371/journal.pone.0118973

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—New capabilities and interfaces. *Nucleic Acids Research*, *40*(15), e115–e115. https://doi.org/10.1093/nar/gks596

Varveri, C., Maliogka, V. I., & Kapari-Isaia, T. (2015). Principles for Supplying Virus-Tested Material. In *Advances in Virus Research* (Vol. 91, pp. 1–32). Elsevier. https://doi.org/10.1016/bs.aivir.2014.10.004

Vega, J., Arahana B., V. S., & Torres, M. D. L. (2015). Estandarización de un protocolo de regeneración de cebolla chalote (Allium cepa var. Aggregatum) a partir de meristemas apicales. *ACI Avances en Ciencias e Ingenierías, 7*(1). https://doi.org/10.18272/aci.v7i1.225

Velásquez-Valle, R., Reveles-Hernández, M., Ileana, Y., Medinaveitia, C.-, & Reveles-Torres, L. R. (2016). *Efecto del tratamiento térmico sobre la presencia de virus en bulbos de ajo (Allium sativum L.).*

Velásquez-Valle, R., Zacatecas, C., & Chew-Madinaveitia, I. (2010). *Presencia de Virus en el Cultivo de Ajo (Allium sativum L.) en Zacatecas, México Virus Presence in the Cultivation of Garlic (Allium sativum L.) in Zacatecas, México*.

Vélez, S. M., Vélez, J. B., & Cedeño, J. J. (2014). *CARACTERÍSTICAS DE LOS VIRUS ASOCIADOS A LAS VIROSIS EN AJO, CEBOLLA Y PIMIENTO EN EL ECUADOR.*

Verbeek, M., Van Dijk, P., & Van Well, P. M. A. (1995). Efficiency of eradication of four viruses from garlic (Allium sativum) by meristem-tip culture. *European Journal of Plant Pathology*, *101*(3), 231–239. https://doi.org/10.1007/BF01874779

Vivek, M., & Modgil, M. (2018). Elimination of viruses through thermotherapy and meristem culture in apple cultivar 'Oregon Spur-II.' *VirusDisease*, *29*(1), 75–82. https://doi.org/10.1007/s13337-018-0437-5

Walkey, D. G. A., Webb, M. J. W., Bolland, C. J., & Miller, A. (1987). Production of virus-free garlic (*Allium sativum* L.) and shallot (*A. ascalonicum* L.) by meristem-tip culture. *Journal of Horticultural Science*, *62*(2), 211–220. https://doi.org/10.1080/14620316.1987.11515771

Wang, M., Hamborg, Z., Blystad, D., & Wang, Q. (2021). Combining thermotherapy with meristem culture for improved eradication of onion yellow dwarf virus and shallot latent virus from infected in vitro-cultured shallot shoots. *Annals of Applied Biology*, *178*(3), 442–449. https://doi.org/10.1111/aab.12646

Wang, M.-R., Cui, Z.-H., Li, J.-W., Hao, X.-Y., Zhao, L., & Wang, Q.-C. (2018). In vitro thermotherapy-based methods for plant virus eradication. *Plant Methods*, *14*(1), 87. https://doi.org/10.1186/s13007-018-0355-y

Wang, M.-R., Zhang, Z., Zámečník, J., Bilavčík, A., Blystad, D.-R., Haugslien, S., & Wang, Q.-C. (2020). Droplet-vitrification for shoot tip cryopreservation of shallot (Allium cepa var. aggregatum): Effects of PVS3 and PVS2 on shoot regrowth. *Plant Cell*,

Tissue and Organ Culture (PCTOC), *140*(1), 185–195. https://doi.org/10.1007/s11240-019-01721-4

Wang, Y. Z., Su, Y., Li, X. Y., Zhang, C. Y., Ma, J. F., Liu, J. Q., Zhang, J. H., Li, J. P., Li, Q. Y., & Zhou, X. P. (2019). First Report of Shallot Virus X and Shallot Latent Virus on Shallot (*Allium cepa* var. *Aggregatum*) in China. *Plant Disease*, *103*(11), 2972–2972. https://doi.org/10.1094/PDIS-04-19-0695-PDN

Ward, L. I., Perez-Egusquiza, Z., Fletcher, J. D., & Clover, G. R. G. (2009). A survey of viral diseases of *Allium* crops in New Zealand. *Australasian Plant Pathology*, *38*(5), 533. https://doi.org/10.1071/AP09039

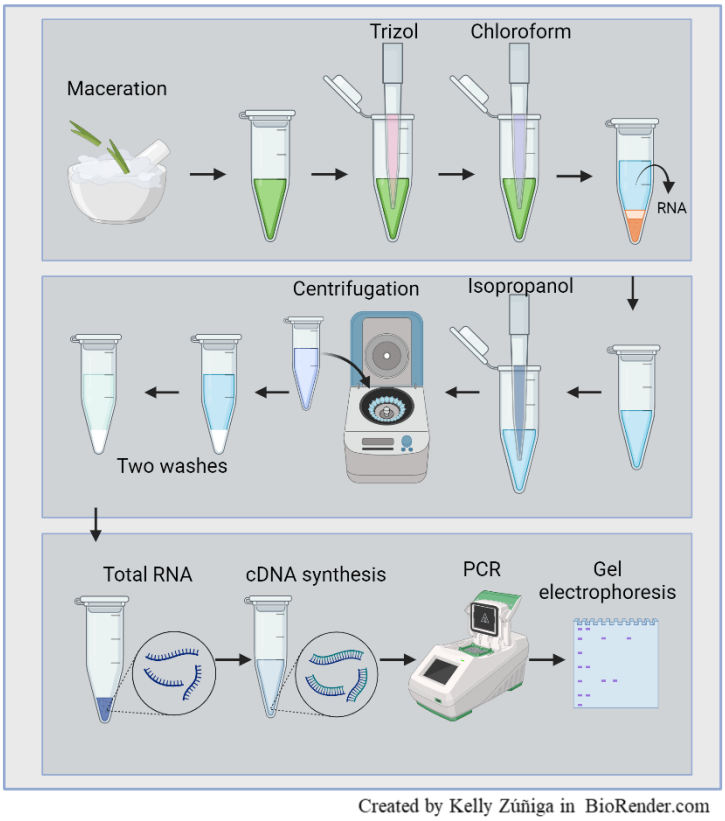
Weilner, S., & Bedlan, G. (2013). Detection of Iris yellow spot virus (IYSV) in selected Allium species and overwintering hosts in Austrian onion-producing areas. *Journal Fur Kulturpflanzen*, *65*, 60–67. https://doi.org/10.5073/JfK.2013.02.03

Zhao, J., Xu, J., Chen, B., Cui, W., Zhou, Z., Song, X., Chen, Z., Zheng, H., Lin, L., Peng, J., Lu, Y., Deng, Z., Chen, J., & Yan, F. (2019). Characterization of Proteins Involved in Chloroplast Targeting Disturbed by Rice Stripe Virus by Novel Protoplast–Chloroplast Proteomics. *International Journal of Molecular Sciences*, *20*(2), 253. https://doi.org/10.3390/ijms20020253

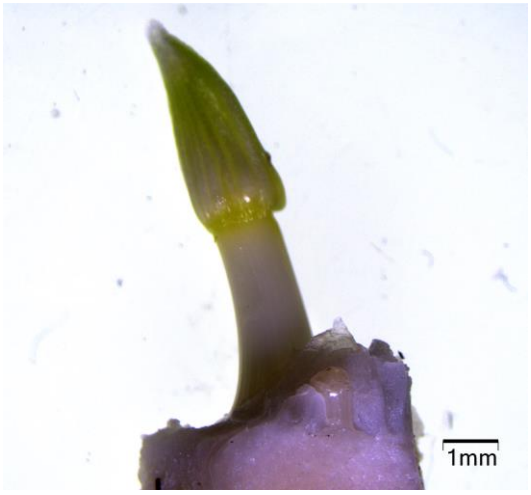
SUPPLEMENTARY FIGURES INDEX

SUPPLEMENTARY TABLES INDEX

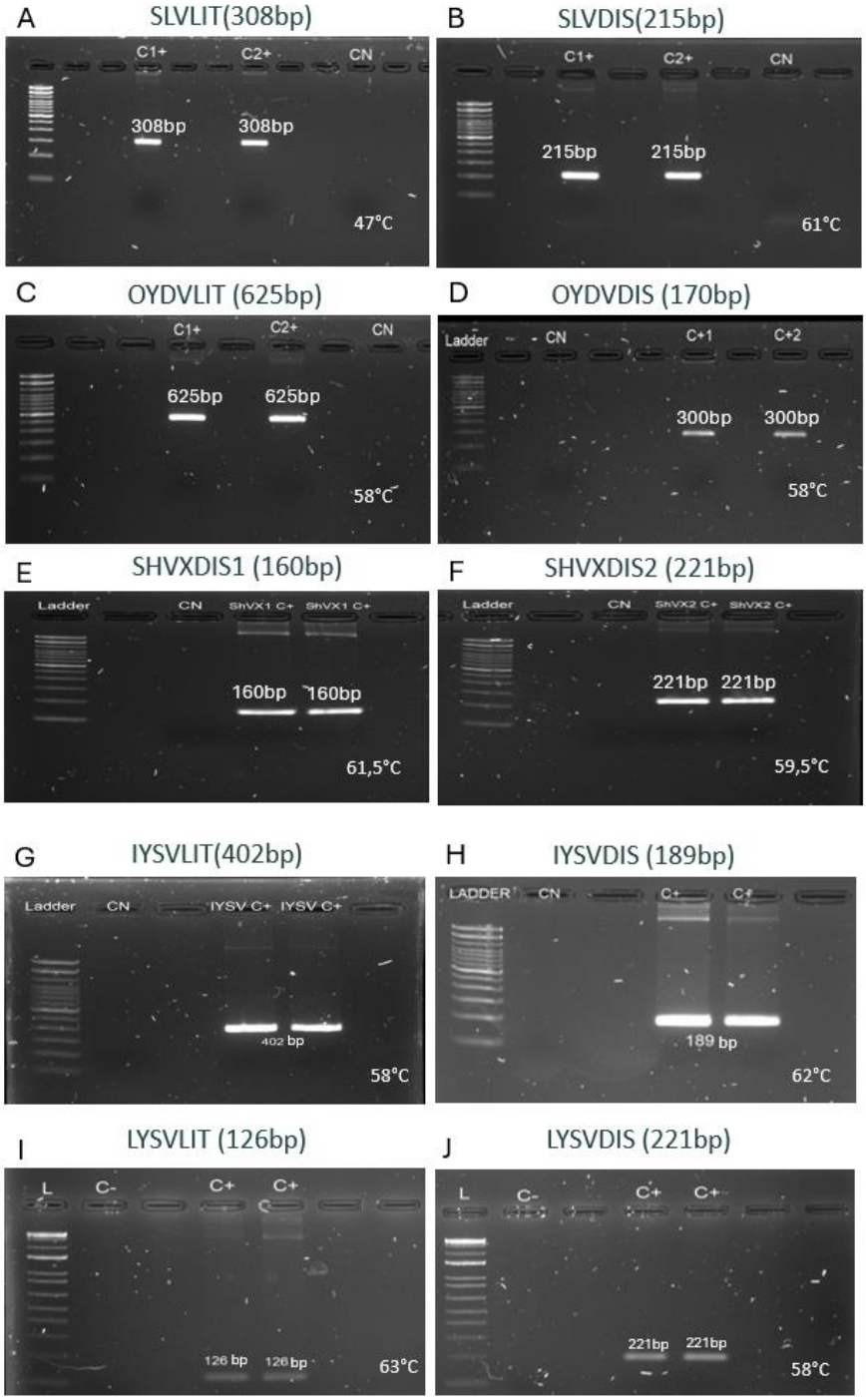
SUPPLEMENTARY MATERIAL



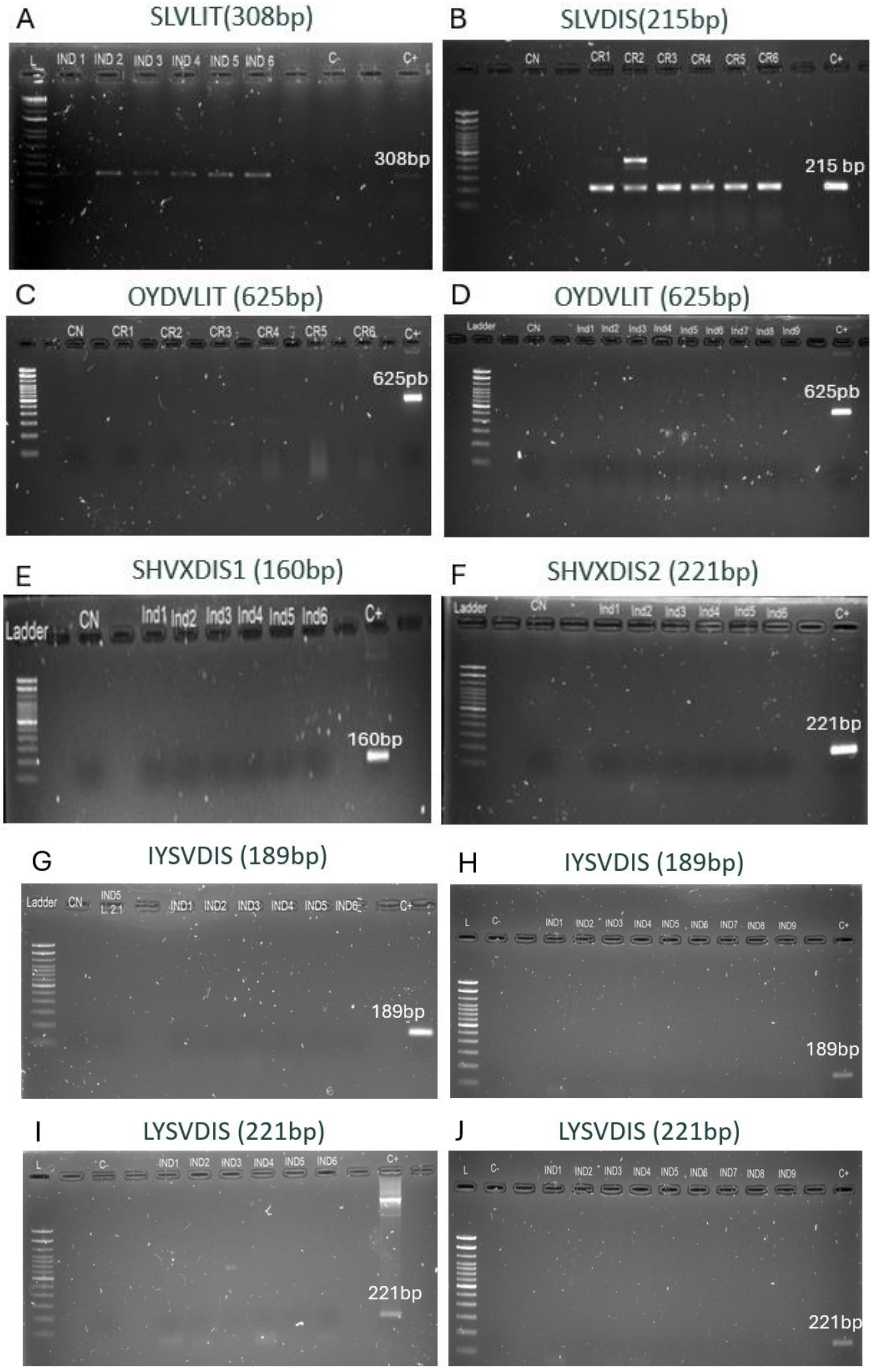
Supplementary Figure 1. Molecular viral detection process. RNA extraction with trizol-chloroform protocol, cDNA synthesis and PCR viral detection.



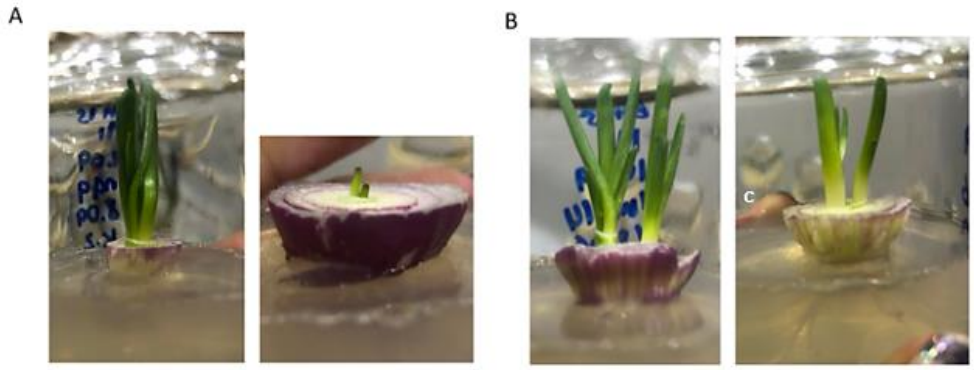
Supplementary Figure 2. Shoot tip with leaf primordia used in shoot tip culture.



Supplementary Figure 3. Amplification of PCR products of positive controls with designed and literature-based primers. **A.** PCR products of SLVLIT with positive controls. **B.** PCR products of SLVDIS with positive controls. **C.** PCR products of OYDVLIT with positive controls. **D.** PCR products of OYDVDIS with positive controls. **E.** PCR products of ShVXDIS1 with positive controls. **F.** PCR products of ShVXDIS2 with positive controls. **G.** PCR products of IYSVLIT with positive controls. **H.** PCR products of IYSVDIS with positive controls. **I.** PCR products from LYSVLIT with positive controls. **J.** PCR products of LYSVDIS with positive controls. The lack of a band implies the absence of the virus. Abbreviations: L: ladder, C+: positive control.



Supplementary Figure 4. Evaluation of PCR products of shallot samples with designed and literature-based primers. **A.** PCR products of SLV with literature primers. **B.** PCR products of SLV with design primers. **C.** PCR products of OYDV with literature primers. **D.** PCR products of OYDV with literature primers. **E.** PCR products of ShVX with literature primers. **F.** PCR products of ShVX with literature primers. **G.** PCR products of IYSV with literature primers. **H.** PCR products of IYSV with literature primers. **I.** PCR products of LYSV with literature primers. **J.** PCR products of LYSV with literature primers. The lack of a band implies the absence of the virus. Abbreviations: L: ladder, C+: positive control.



Supplementary Figure 5. Sprouting results of basal discs at 15 days of in vitro culture . A. Control group. B. Treatment group.

Supplementary Table 1. Disinfection protocol for shallot bulbs

Group	Washing time with Mertect fungicide (min)	Chemical disinfection protocol	Initial culture media
Treatment	3,5 g/L for 15 minutes	Et-OH (70%) for 10 min	M1: MS + NAA + BAP
		NaClO (4,5%) + Tween-20 for 35 min	
		Washes with sterile dH2O	
Control	No washing	Et-OH (70%) for 10 min	M1: MS + ANA + BAP
		NaClO (4,5%) + Tween-20 for 30 min	
		Washes with sterile dH2O	

Supplementary Table 2. Media composition obtained from Vega et al. (2015).

Medium	Composition
Murashige & Skoog (MS)	100ml Major Elements, 10ml Minor Elements, 50ml Organic Elements, 10ml FeDA, sucrose 30 g/L, and agar 8 g/L
M1	MS medium, NAA (1.1 µM), BAP (8.9 µM), sucrose 30 g/L, and agar 8 g/L
M2	MS medium
M3	MS medium, NaPO4 300 mg/L, ancymidol 10 µM, sucrose 50 g/L, and agar 8 g/L

Supplementary Table 3. Quantification of total RNA extracted from shallot leaves and absorbance index before *in vitro* culture.

Sample	Experimental farm			Organic market			Iñaquito market 1			Iñaquito market 2		
	ng/µL	260/280	260/230	ng/µL	260/280	260/230	ng/µL	260/280	260/230	ng/µL	260/280	260/230
1	764,00	2,01	2,43	616,00	1,90	0,83	590,00	2,01	1,60	636,00	1,72	0,40
2	554,00	2,03	2,18	448,00	1,78	1,07	588,00	1,95	0,91	196,00	1,89	1,34
3	386,00	1,97	2,31	748,00	1,98	1,26	626,00	1,85	0,65	214,00	1,95	1,39
4	686,00	1,97	2,01	608,00	1,99	1,77	101,00	1,91	0,41	364,00	1,94	0,96
5	768,00	1,94	2,36	798,00	1,84	0,66	732,00	2,01	1,48	454,00	2,03	1,83
6	324,00	1,96	1,25	866,00	2,01	1,24	276,00	1,95	0,56	121,00	1,82	0,42
7	800,00	2,04	2,26	892,00	2,05	1,32	474,00	2,02	1,39	149,00	1,87	0,75
8	738,00	2,04	2,36	546,00	1,94	0,99	1080,00	2,06	1,14	110,00	1,76	0,31
9	430,00	1,98	1,36	656,00	1,94	0,99	286,00	1,88	0,78	199,00	1,95	1,94
10	656,00	2,01	2,29	200,00	1,92	1,98	382,00	1,98	1,50	486,00	2,01	2,03
11	328,00	1,95	2,21	324,00	1,98	2,21	226,00	1,99	1,56	234,00	2,01	1,21
12	404,00	1,89	1,76	264,00	1,98	2,03	76,00	1,77	0,64	149,00	2,01	1,23
13	994,00	2,05	2,34	172,00	1,91	1,02	1200,00	2,01	2,15	234,00	1,98	2,02
14	426,00	2,08	2,33	904,00	2,02	2,44	62,20	1,97	1,03	462,00	1,97	1,74
15	426,00	1,97	2,27	272,00	1,93	1,85	177,00	1,89	1,43	264,00	1,96	1,78
16	526,00	1,92	2,12	234,00	1,98	1,90	127,00	1,89	0,35	338,00	1,94	2,06
17	320,00	1,91	1,82	164,00	1,98	1,53	814,00	1,94	1,43	220,00	1,99	0,83
18	808,00	2,01	2,29	41.6	1,83	0,52	660,00	2,05	2,37	80,00	2,88	0,89
19	438,00	1,93	1,82	324,00	1,91	0,47	578,00	1,87	1,53	302,00	1,99	1,23
20	740,00	1,94	2,44	440,00	1,97	1,10	602,00	1,96	2,21	-*	1,97	0,81
21	466,00	1,91	1,76	630,00	2,03	1,35	812,00	2,01	2,33	256,00	1,85	0,58
22	280,00	1,94	1,50	410,00	1,98	1,10	412,00	1,99	2,36	80,60	3,23	0,71
23	438,00	1,97	2,03	1020,00	1,97	1,97	970,00	1,98	2,32	318,00	1,87	1,14
24	-	-	-	514,00	1,71	1,09	302,00	1,91	0,64	185,00	1,76	0,24
25	-	-	-	562,00	1,79	1,33	192,00	1,84	1,07	222,00	1,82	0,93
26	-	-	-	185,00	1,74	0,86	-	-	-	-	-	-
Mean	**552,20**	**1,98**	**2,07**	**508,20**	**1,93**	**1,34**	**493,80**	**1,95**	**1,35**	**261,40**	**2,01**	**1,15**
S	**199,20**	**0,05**	**0,35**	**254,10**	**0,09**	**0,53**	**320,90**	**0,07**	**0,65**	**138,90**	**0,33**	**0,58**
Min	**280,00**	**1,89**	**1,25**	**164,00**	**1,71**	**0,47**	**62,20**	**1,77**	**0,35**	**80,00**	**1,72**	**0,24**
Max	**994,00**	**2,08**	**2,44**	**1020,00**	**2,05**	**2,44**	**1200,00**	**2,06**	**2,37**	**636,00**	**3,23**	**2,06**

* Low concentration

Supplementary Table 4. Quantification of total RNA extracted from shallot leaves and absorbance index after *in vitro* culture.

Sample	Trial 1			Trial 2			Trial 3		
	ng/µL	**260/280**	**260/230**	**ng/µL**	**260/280**	**260/230**	**ng/µL**	**260/280**	**260/230**
1	798	1,99	2,38	650	1,97	1,96	638	2,08	1,78
2	506	1,97	2,2	1180	2,00	2,3	426	2,05	2,39
3	764	2,03	2,33	802	1,97	1,92	832	2,07	1,77
4	826	1,03	2,46	1080	2,08	2,38	328	2,07	2,37
5	750	2,03	2,31	1060	2,08	2,43	1120	1,98	1,84
6	1100	2,01	2,4	1100	2,08	2,2	248	1,61	0,29
7	1020	2,00	2,00	802	2,02	2,22	-*	1,55	0,14
8	1100	2,02	2,16	610	2,00	2,21	1140	2,02	2,02
9	364	2,04	2,47	-	-	-	1080	1,97	2,38
10	258	2,06	2,4	-	-	-	616	1,78	1,42
11	118	2,04	2,35	-	-	-	712	2,04	2,3
12	-	-	-	-	-	-	808	2,04	2,22
Mean	**691,30**	**1,93**	**2,32**	**910,50**	**2,03**	**2,20**	**722,50**	**1,94**	**1,74**
SD	**337,40**	**0,30**	**0,14**	**220,80**	**0,05**	**0,18**	**310,90**	**0,19**	**0,78**
Max	**118,00**	**2,06**	**2,47**	**610,00**	**1,97**	**1,92**	**248,00**	**1,55**	**0,14**
Min	**1100,00**	**1,03**	**2,00**	**1180,00**	**2,08**	**2,43**	**1140,00**	**2,08**	**2,39**

* Low concentration

Supplementary Table 5. Viral molecular detection of shallots from the USFQ experimental farm before *in vitro* culture.

Sample	RNA (Qubit)	Virus				
		SLV	OYDV	IYSV	ShVX	LYSV
		LIT	LIT	DIS	DIS 1	DIS
1	764	Positive	Negative	Negative	Negative	Negative
2	554	Positive	Negative	Negative	Negative	Negative
3	386	Positive	Negative	Negative	Negative	Negative
4	686	Positive	Negative	Negative	Negative	Negative
5	768	Positive	Negative	Negative	Negative	Negative
6	324	Positive	Negative	Negative	Negative	Negative
7	800	Positive	Negative	Negative	Negative	Negative
8	738	Positive	Negative	Negative	Negative	Negative
9	430	Positive	Negative	Negative	Negative	Negative
10	656	Positive	Negative	Negative	Negative	Negative
11	328	Positive	Negative	Negative	Negative	Negative
12	404	Positive	Negative	Negative	Negative	Negative
13	994	Positive	Negative	Negative	Negative	Negative
14	426	Positive	Negative	Negative	Negative	Negative
15	426	Positive	Negative	Negative	Negative	Negative
16	526	Positive	Negative	Negative	Negative	Negative
17	320	Positive	Negative	Negative	Negative	Negative
18	808	Positive	Negative	Negative	Negative	Negative
19	438	Positive	Negative	Negative	Negative	Negative
20	740	Positive	Negative	Negative	Negative	Negative
21	466	Positive	Negative	Negative	Negative	Negative
22	280	Positive	Negative	Negative	Negative	Negative
23	438	Positive	Negative	Negative	Negative	Negative

Abbreviation: SLV, Shallot Latent Virus; OYDV, Onion Yellow Dwarf Virus; IYSV, Iris Yellow Spot Virus; ShVX, Shallot Virus X; and LYSV, Leek Yellow Stripe Virus.

Supplementary Table 6. Molecular detection of shallots belonging to organic market before *in vitro* culture.

Sample	RNA (Qubit)	Virus				
		SLV	**OYDV**	**IYSV**	**ShVX**	**LYSV**
		LIT	**LIT**	**DIS**	**DIS 1**	**DIS**
1	616	Positive	Negative	Positive	Negative	Negative
2	448	Positive	Negative	Positive	Negative	Negative
3	748	Positive	Negative	Positive	Negative	Negative
4	608	Positive	Negative	Negative	Negative	Negative
5	798	Positive	Negative	Negative	Negative	Negative
6	866	Positive	Negative	Negative	Negative	Negative
7	892	Positive	Negative	Negative	Negative	Negative
8	546	Positive	Negative	Negative	Negative	Negative
9	656	Positive	Negative	Negative	Negative	Negative
10	200	Positive	Negative	Negative	Negative	Negative
11	324	Positive	Negative	Negative	Negative	Negative
12	264	Positive	Negative	Negative	Negative	Negative
13	172	Positive	Negative	Negative	Negative	Negative
14	904	Positive	Negative	Negative	Negative	Negative
15	272	Positive	Negative	Negative	Negative	Negative
16	234	Positive	Negative	Negative	Negative	Negative
17	164	Positive	Negative	Positive	Negative	Negative
18	41.6	Positive	Negative	Negative	Negative	Negative
19	324	Positive	Negative	Negative	Negative	Negative
20	440	Positive	Negative	Negative	Negative	Negative
21	630	Positive	Negative	Negative	Negative	Negative
22	410	Positive	Negative	Negative	Negative	Negative
23	1020	Positive	Negative	Negative	Negative	Negative
24	514	Positive	Negative	Negative	Negative	Negative
25	562	Positive	Negative	Negative	Negative	Negative
26	185	Positive	Negative	Positive	Negative	Negative

Abbreviation: SLV, Shallot Latent Virus; OYDV, Onion Yellow Dwarf Virus; IYSV, Iris Yellow Spot Virus; ShVX, Shallot Virus X; and LYSV, Leek Yellow Stripe Virus.

Supplementary Table 7. Molecular detection of shallots belonging to Iñaquito 1 (first supplier) before *in vitro* culture.

Sample	ARN (Qubit)	Virus (Primer)				
		SLV	OYDV	IYSV	ShVX	LYSV
		LIT	LIT	DIS	DIS 1	DIS
1	590	Positive	Negative	Negative	Negative	Negative
2	588	Negative	Negative	Negative	Negative	Negative
3	626	Positive	Negative	Positive	Negative	Negative
4	101	Negative	Negative	Negative	Negative	Negative
5	732	Positive	Negative	Negative	Negative	Negative
6	276	Positive	Negative	Negative	Negative	Negative
7	474	Positive	Negative	Positive	Negative	Negative
8	1080	Positive	Negative	Negative	Negative	Negative
9	286	Positive	Negative	Negative	Negative	Negative
10	382	Positive	Negative	Negative	Negative	Negative
11	226	Negative	Negative	Positive	Negative	Negative
12	76	Negative	Negative	Negative	Negative	Negative
13	1200	Positive	Negative	Negative	Negative	Negative
14	62,2	Positive	Negative	Negative	Negative	Negative
15	177	Positive	Negative	Negative	Negative	Negative
16	127	Negative	Negative	Negative	Negative	Negative
17	814	Negative	Negative	Positive	Negative	Negative
18	660	Negative	Negative	Negative	Negative	Negative
19	578	Positive	Negative	Positive	Negative	Negative
20	602	Negative	Negative	Negative	Negative	Negative
21	812	Negative	Negative	Negative	Negative	Negative
22	412	Negative	Negative	Negative	Negative	Negative
23	970	Negative	Negative	Negative	Negative	Negative
24	302	Positive	Negative	Negative	Negative	Negative
25	192	Positive	Negative	Positive	Negative	Negative

Abbreviation: SLV, Shallot Latent Virus; OYDV, Onion Yellow Dwarf Virus; IYSV, Iris Yellow Spot Virus; ShVX, Shallot Virus X; and LYSV, Leek Yellow Stripe Virus.

Supplementary Table 8. Molecular detection of shallots belonging to the Iñaquito 2 (second supplier) before *in vitro* culture.

Sample	ARN (Qubit)	Virus				
		SLV	**OYDV**	**IYSV**	**ShVX**	**LYSV**
		LIT	**LIT**	**DIS**	**DIS 1**	**DIS**
1	636	Positive	Negative	Negative	Negative	Negative
2	196	Positive	Negative	Negative	Negative	Negative
3	214	Positive	Negative	Positive	Negative	Negative
4	364	Positive	Negative	Positive	Negative	Negative
5	454	Positive	Negative	Positive	Negative	Negative
6	121	Positive	Negative	Positive	Negative	Negative
7	149	Positive	Negative	Positive	Negative	Negative
8	110	Positive	Negative	Positive	Negative	Negative
9	199	Positive	Negative	Positive	Negative	Negative
10	486	Positive	Negative	Positive	Negative	Negative
11	234	Positive	Negative	Positive	Negative	Negative
12	149	Positive	Negative	Positive	Negative	Negative
13	234	Positive	Negative	Positive	Negative	Negative
14	462	Positive	Negative	Negative	Negative	Negative
15	264	Positive	Negative	Negative	Negative	Negative
16	338	Positive	Negative	Positive	Negative	Negative
17	220	Positive	Negative	Negative	Negative	Negative
18	80	Positive	Negative	Positive	Negative	Negative
19	302	Positive	Negative	Positive	Negative	Negative
20	-*	Positive	Negative	Negative	Negative	Negative
21	256	Positive	Negative	Positive	Negative	Negative
22	80,6	Negative	Positive	Negative	Negative	Negative
23	318	Positive	Negative	Positive	Negative	Negative
24	185	Positive	Negative	Positive	Negative	Negative
25	222	Positive	Negative	Positive	Negative	Negative

Abbreviation: SLV, Shallot Latent Virus; OYDV, Onion Yellow Dwarf Virus; IYSV, Iris Yellow Spot Virus; ShVX, Shallot Virus X; and LYSV, Leek Yellow Stripe Virus.
*Low concentration.