

Tomato spotted wilt virus detection in the potato cultivar Innovator

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ABSTRACT

Tomato spotted wilt orthotospovirus (TSWV) causes important economic losses in potato production. The cultivar Innovator is the most widely used variety for potato industrial processing and the second most extensively grown cultivar in Argentina. The objective of this study was to identify the most suitable tissue sample for TSWV detection in potato cv. Innovator using DAS-ELISA. Tissue samples taken from three different points of the tuber and from the leaves of seedlings originating from two types of sprouts were evaluated for TSWV detection using DAS-ELISA. Detection of TSWV in the tuber tissue was 95%, 86% and 87% for the sample taken below the rose end sprout, below the lateral end sprout, and the pith, respectively. Detection of TSWV in seedlings tissue was 41% and 40% in plants grown from rose and lateral sprouts, respectively. The proportion of positive TSWV samples was higher in samples collected from infected tubers than in those collected from seedlings. Therefore, tuber tissue proved to be suitable for TSWV detection by DAS-ELISA, regardless of the sampling point, because there were no significant differences among points. Sampling the tuber tissue reduces sample collection time since it is not necessary to obtain the progeny plant to analyze the leaves. TSWV caused severe damage in 46% of the infected tubers, which presented symptoms of internal necrosis, reducing the quality and the commercial value of the potato cv. Innovator. The application of an accurate TSWV diagnostic technique is required to achieve an efficient and effective management of this disease in potato. In this context, the inclusion of TSWV detection in the Argentine potato seed certification program would be a positive measure.

Keywords: Orthotospovirus, TSWV detection in tuber, seed-potato certification.

RESUMEN

Tomato spotted wilt orthotospovirus (TSWV) causa importantes pérdidas económicas en la producción de papa. El cultivar Innovator es el más utilizado en el procesamiento industrial de papa y la segunda variedad más cultivada en Argentina. El objetivo de este trabajo fue determinar el tejido más adecuado para la detección de TSWV mediante DAS-ELISA en papa cv. Innovator. Muestras de tejido de tres diferentes puntos del tubérculo y de hojas de plántulas desarrolladas a partir de dos tipos de brote fueron evaluados para detectar TSWV mediante DAS-ELISA. La detección de TSWV en el tubérculo fue del 95%, 86%, 87% para el tejido debajo del brote apical, para el tejido debajo del brote lateral y para el tejido de la médula, respectivamente. La detección de TSWV fue del 41% y 40% en plantas cultivadas a partir del brote apical y lateral, respectivamente. La proporción de muestras positivas para TSWV fue mayor en las muestras recolectadas de tubérculos infectados que en las de plantas de la progenie para el cv. Innovator. Por lo tanto, el tejido

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del tubérculo resultó ser adecuado para la detección de TSWV mediante DAS-ELISA independientemente del punto de muestreo porque no se encontraron diferencias significativas entre ellos. El muestreo del tejido del tubérculo reduce el tiempo de recolección de las muestras debido a que no es necesario obtener la planta de la progenie para analizar las hojas. TSWV ocasionó daños severos en el 46% de los tubérculos infectados, los cuales presentaron síntomas de necrosis interna, reduciendo la calidad y el valor comercial de la papa cv. Innovator. La aplicación de técnicas precisas de diagnóstico de TSWV es necesaria para lograr un manejo eficiente y efectivo de esta enfermedad en papa. En este contexto, la inclusión de la detección de TSWV en el programa argentino de certificación de papa semilla podría ser una medida positiva.

Palabras clave: *Orthotospovirus*, detección de TSWV en tubérculos, certificación de papa-semilla.

INTRODUCTION

Orthotospovirus tomatomaculæ, hereafter referred to as TSWV (tomato spotted wilt orthotospovirus), is a member of the genus *Orthotospovirus*, family *Tospoviridae*, order *Bunyvirales* (Abudurexiti *et al.*, 2019; Maes *et al.*, 2019). TSWV is one of the viruses of greatest economic importance, causing severe losses in many crops worldwide, including bean, chrysanthemum, papaya, peanut, pepper, potato, tobacco, and tomato (Rybicki, 2015; Oliver and Whitfield, 2016; Pappu *et al.*, 2009; Scholthof *et al.*, 2011). This virus has a wide host range, infecting at least 1090 species belonging to 70 botanical families, both monocots and dicots (Parrella *et al.*, 2003; Hanssen *et al.*, 2010). There are no records of TSWV transmission by botanical seed or pollen. TSWV is transmitted by thrips species of the order *Thysanoptera* in a persistent and circulative-propagative manner (Rotenberg *et al.*, 2015). In Argentina, four of the nine thrips species reported to transmit TSWV were identified as follows: *Frankliniella occidentalis* (Pergande), *Frankliniella schultzei* (Tribom), *Thrips tabaci* (Lindemann) and *Frankliniella gemina* (Bagnall) (De Borbón *et al.*, 1999; De Borbón *et al.*, 2006; Salvalaggio *et al.*, 2017). In potato, the disease caused by TSWV is known as “top necrosis” and produces necrotic symptoms in leaves, stems, and tubers (Bulajić *et al.*, 2014). Tubers produced by infected plants may appear healthy or may be deformed, with cracks and internal rusty or dark necrotic spots. These spots become visible through the skin as concentric patterns or inside when the tuber is cut. Reduction in tuber size was also reported (Costa and Hooker, 1980; Abad *et al.*, 2005).

TSWV affecting potato was first reported in Brazil in 1938 and in Argentina in 1944 (Costa and Kiehl, 1938; Pontis and Feldman, 1962). There is a differential geographic distribution of *Orthotospovirus* species in potato crops in Argentina. TSWV is present in the Buenos Aires and Mendoza potato producing regions, while groundnut ringspot orthotospovirus (*Orthotospovirus arachianuli*) is prevalent in the Córdoba producing areas (López Lambertini, 2018). TSWV has spread in potato crops, producing severe economic losses in the south-east of Buenos Aires province in 2008/2009 (Escarrá *et al.*, 2004). The most severe damage was reported on the cv. Innovator (Carrizo *et al.*, 2010). The industry demands 30% of the total potato production and the province of Buenos Aires contributes 82%. Innovator (Shepody x RZ-84-2580, HZPC Americas corporation) is the most widely used cultivar for production of chips or french fries and stands out for its high dry matter content (Carrizo *et al.*, 2010; Saldrigas, 2018; The European Cultivated Potato Database, 2020).

Potatoes are primarily propagated vegetatively via tubers; therefore, the virus accumulates in planting material, causing a reduction in yield or quality. This phenomenon is known as seed potato degeneration (Thomas-Sharma *et al.*, 2016). The use of certificated seed that are virus-free has reduced or eliminated these pathogens significantly (Frost *et al.*, 2013; Onofre, *et al.*, 2021). Bulajić *et al.* (2014) showed that TSWV transmission rates from plants to tubers and from infected tubers to progeny are affected by the cultivar rather than by the TSWV isolate. Wilson (2001) found that TSWV has an erratic distribution in tubers, which could affect the reliability of detection using ELISA. The main objective of this study was to identify the most suitable tissue sample for TSWV detection in potato cv. Innovator using DAS-ELISA.

MATERIALS AND METHODS

Plants naturally infected with TSWV

Eighty-six cv. Innovator potato plants developed from seed potatoes certified as free from PVY, PLRV and PVX with typical TSWV symptoms were selected at the end of flowering (50 days after planting) from a cultivated field in Balcarce (southeast of Buenos Aires province, Argentina). Then, the collected leaves were taken to the laboratory and analysed using DAS-ELISA (Double-Antibody Sandwich Enzyme-Linked Immunosorbent Assay) to confirm TSWV infection.

Evaluation of tuber tissue for TSWV detection

One tuber was randomly selected and harvested from each TSWV-infected plant at the end of the growing season. Tubers were treated with Rindite to break dormancy and then stored at 25 °C and at relative humidity of 80-90% in the dark (Resolution SAGPYA 217, 2002). From each of the 86 tubers, tissue samples were taken from three points: the base of the rose end sprout, the base of lateral sprouts and the pith 21 days after the treatment. These samples were tested for TSWV presence by DAS-ELISA.

Evaluation of seedling leaf for TSWV detection

Eye plugs from the rose end and lateral end sprouts of each tuber were removed using a melon scoop 21 days after the Rindite treatment, planted in individual pots and grown in an insect-free greenhouse for about 3 to 4 weeks (Oliver and Whitfield, 2016). The presence of disease symptoms in seedlings

was recorded 4 weeks after planting. After 3 to 4 weeks of growth, the first fully expanded apical leaf was collected from each progeny plant and tested for TSWV by DAS-ELISA.

TSWV detection

The presence of TSWV in different tissues was evaluated using DAS-ELISA. Microtiter plates (NUNC MaxiSorp) were coated with anti-TSWV antibodies (Bioreba, Switzerland) 1: 1000 (v/v) in carbonate buffer at pH 9.6 (Clark and Adams, 1977). ELISA plates were incubated overnight at 5 °C, followed by three washings. Plant material was extracted using a Tecan AG 450 sap extractor and a dilution of 1:20 (w/v) for tuber samples and 1:10 (weight/volume) for leaf samples in phosphate buffer (pH 7.4) containing PVP 20 g/L added to plates. After overnight incubation at 5°C and three washing steps, the plates were incubated with anti-TSWV IgG alkaline phosphatase conjugate (Bioreba, Switzerland) diluted 1:1000 at 37 °C for 4 h. Finally, the plates were washed and p-nitrophenyl phosphate substrate (Sigma-Aldrich Corporation, USA, 1mg mL⁻¹) in 0.1 M the diethanolamine (pH 9.8) was added. ELISA plates were read after 30 minutes using a Tecan Sunrise microplate reader at 450 nm. Two positive controls from frozen leaf tissues infected with TSWV and three negative controls from healthy potato tuber tissues were included in the plates. Positive samples were those whose absorbance value was equal to or higher than the mean of absorbance of healthy controls plus three standard deviations.

Statistical analysis

The percentages of TSWV positive samples by DAS-ELISA were calculated for each sampling point in the tuber (below the rose end and lateral end sprouts, and in the pith) and seedling

type (originating from the rose or lateral sprouts). A Pearson chi-square independence test was performed to evaluate the association between the origin of the tuber sample and the presence of TSWV. The significance level was 0.05. In addition, a confidence interval of 0.95 was estimated for each sampling point in the tuber and for each type of seedling (Agresti, 2002).

RESULTS

All the symptomatic potato plants cv. Innovator selected in the potato field production tested positive for TSWV by DAS-ELISA and presented necrotic patches or spots and necrotic concentric rings on the leaves (fig. 1 a). In addition, of 86 progeny tubers that resulted positive by DAS-ELISA, 40 (46%) presented internal necrosis, which reduced the quality and commercial value of the potato crop (fig. 1 c and d).

In the tuber, TSWV was detected in tissue sampled in at least one of the three points (rose end sprout, lateral end sprout and pith). The association between the tuber sampling points and positive TSWV samples detected by DAS-ELISA was not statistically significant ($p=0.0893$). Likewise, the detection of TSWV in one sampling point was not associated with the detection of TSWV in another sampling point for the same tuber. Detection of TSWV in the tuber tissue was 95%, 86% and 87% for the sample taken below the rose end sprout, below the lateral end sprout, and the pith, respectively (table 1). The TSWV detection percentages obtained did not differ statistically ($p\geq 0.05$) among the sampling points in potato tuber tissue. This result shows that the tissue taken from the three sampling points in the tuber was suitable for TSWV detection by DAS-ELISA in cv. Innovator (table 1). Consequently, the distribution of TSWV in the tuber seems to be uniform.

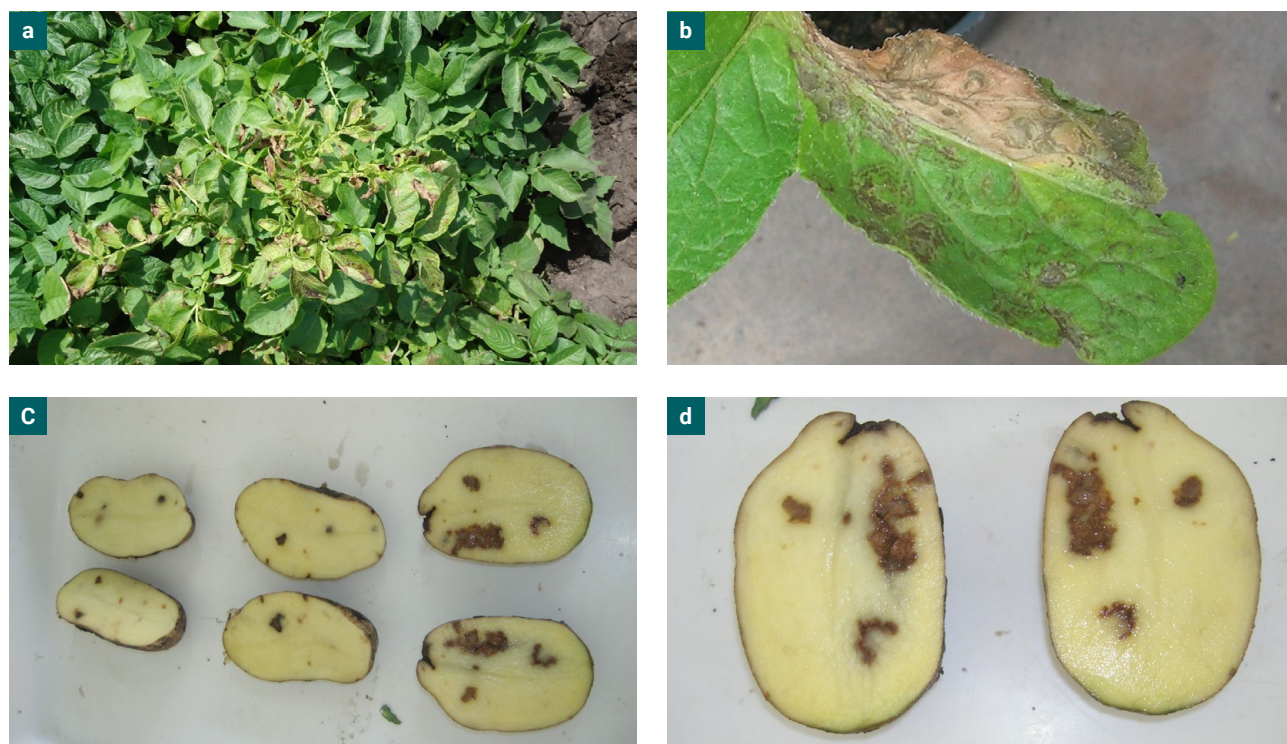


Figure 1. Symptoms of TSWV in potato cv. Innovator: a) necrotic patches and spots on leaves; b) concentric rings on leaves; c) and d) tubers showing brown flecking.

In the case of progeny plants, not all the seedlings originated from infected tubers resulted positive for TSWV and not all the infected seedlings presented symptoms. The symptoms observed were necrotic spots and rings in leaves and stems (fig. 1 b). TSWV was detected in 41% (28/69) of the leaves of seedlings originating from the rose end sprout (table 1). Of the 28 seedlings positive by DAS-ELISA, 12 were asymptomatic and the remaining 16 presented TSWV symptoms. On the other hand, 40% (30/75) of the leaves originating from the lateral end sprout resulted positive for TSWV (table 1). Of the 30 seedlings positive by DAS-ELISA, 13 were asymptomatic and the remaining 17 presented TSWV symptoms. No symptoms were observed in the TSWV negative seedlings.

The proportion of positive TSWV samples was higher in samples collected from infected tubers than in those collected from the seedlings (figure 2). Therefore, the tuber tissue was suitable for TSWV detection by DAS-ELISA in cv. Innovator. Using tuber tissue reduces sample collection time since it is not necessary to obtain the progeny plant to analyze the leaves.

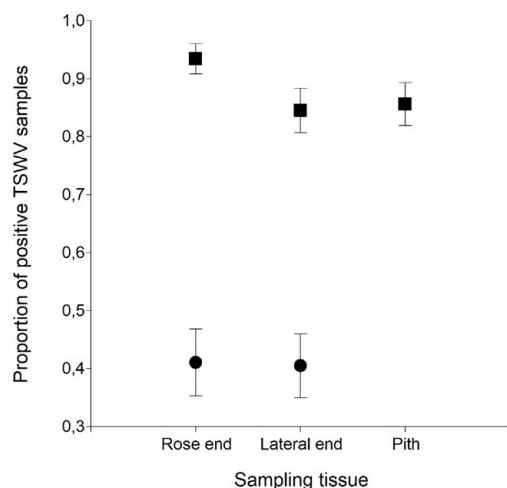


Figure 2. Proportion of positive TSWV detection and standard error in potato cv. Innovator according to the origin of the sample in the tuber: rose end, lateral end, and pith (square), and leaves of seedlings originating from rose end and lateral end sprouts (dot).

DISCUSSION

The TSWV detection percentages did not differ significantly ($p \geq 0.05$) among the tuber sampling points: rose end (95%), lateral end (86%) and pith (87%) in cv. Innovator. These results agree with the findings obtained in other potato cultivars like Riviera, Arnova, Curoda, Kondor and Aladin (Bulajić *et al.*, 2013). In contrast, Wilson (2001) reported variable detection values in the tuber cv. Russet Burbank: 62.5% in rose end, 80% in internal pith, and 25% in heel end. The different results among cultivars shows the importance of establishing the suitable tissue sample for efficient TSWV detection in each potato cultivar. Moreover, the TSWV detection percentage was the same for the leaf of seedlings originating from both the rose sprout and the lateral sprout. However, the proportion of TSWV detection by DAS-ELISA in cv. Innovator was higher in samples collected from infected tubers than those collected from progeny plants; hence, the tuber tissue is the most suitable sample for detecting TSWV.

The difference in detection between infected tubers and seedlings can be attributed to several factors. In this study, early TSWV infections (before 50 days after planting) may have favored virus translocation to the tuber. For PVY, a mature plant resistance phenomenon, i.e. a faster translocation of the virus from the leaves to the progeny tubers in young than in old plants was reported and was attributed mainly to cell-to-cell movement restrictions (Dupuis, 2017). On the other hand, the lower number of TSWV-infected seedlings could be due to the interruption of virus movement after the removal of sprouts from the tuber to obtain the seedling. The fact that not all infected tubers produce infected plants is consistent with previous findings in other cultivars (Wilson, 2001).

In this work, TSWV seems to have a uniform distribution in potato tuber cv. Innovator. The distribution reported for other viruses infecting potato was also even for PVX, but uneven for PVY and PLRV (García Ruíz *et al.*, 2016; Gugerli and Gehriger, 1980; Salazar, 1995, Whitworth *et al.*, 2012). However, in PVY, the maximum virus concentration occurs at the rose end of the tuber and undergoes changes during storage; whereas in PLRV, it occurs at the heel end of the tuber (Fox *et al.*, 2005; Salazar, 1995; Whitworth *et al.*, 2012). The sample used for detecting PVY and PVX was taken from the tissue below the rose end sprout, whereas for PLRV, the tissue below the lateral end sprout is used (Resolution SAGPYA 217, 2002). However,

Sample origin	TSWV DAS-ELISA detection			
	Tuber		Leaf of progeny plant (seedling)	
Rose end	0.95 (82/86) ns	[0.88;0.99]	0.41 (28/69*) ns	[0.30;0.52]
Lateral end	0.86 (74/86) ns	[0.77;0.92]	0.40 (30/75*) ns	[0.30;0.51]
Pith	0.87 (75/86) ns	[0.78;0.93]		ND

ns: statistically non-significant ($P \geq 0.05$) within a column for independences test. ND: not determined. The lower and upper limits of confidence interval values are indicated in square brackets.

*The lower number of progeny plants obtained was due to failure of sprout emergence or sprout death, which could be attributed to TSWV infection.

Table 1. Proportion and confidence interval of positive TSWV samples by DAS-ELISA in potato cv. Innovator according to the origin of the sample: tuber (rose end, lateral end, and pith) and leaf of seedlings originating from two types of sprouts (rose end or lateral end).

TSWV is not included in the Argentine potato seed certification scheme at present. The results of this work show that the tuber tissue below the rose end sprout is suitable for TSWV detection by DAS-ELISA in the Argentine potato seed certification scheme since it is the tissue sample used for PVX and PVY diagnosis.

Overall, our results show the susceptibility of potato cv. Innovator to TSWV and the reduction of tuber quality due to this virus since 46% of the tubers presented internal necrosis (fig. 1). At the same time, 54% of the TSWV-infected tubers did not show virus symptoms. This result warns of the risk of spreading the virus if non-certified seed potato is used for multiplication in Argentina. A study focusing on the temporal and spatial dynamics of TSWV in cv. Innovator showed that the disease is polycyclic and reflects the importance of using certified seed potatoes free from TSWV to reduce inoculum sources that might cause primary infections (Salvalaggio *et al.*, 2017). Finally, using TSWV-certified seed is an efficient strategy to manage this virus since no potato cultivars resistant to TSWV are available.

CONCLUSION

The tuber is a suitable tissue for TSWV detection by DAS-ELISA in cv. Innovator. Using this tissue is also cost-efficient since it is not necessary to obtain the progeny plant to analyze the leaves. TSWV caused severe damage in 46% of the infected tubers, which presented symptoms of internal necrosis, reducing the quality and commercial value of the potato cv. Innovator. The application of an accurate TSWV diagnostic technique is required for efficient and effective management of this disease in potato. In this context, the inclusion of TSWV detection in the Argentine potato seed certification program would be a positive measure.

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