Susceptibility of Solanum phureja (Juz. et Buk.) to potato yellow vein virus

Susceptibilidad de Solanum phureja (Juz. et Buk.) al virus del amarillamiento de las venas

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ABSTRACT
Sixty two virus-free accessions of Solanum phureja from the Colombian central collection of this tuber (Bogotá-Soacha) were tested for their susceptibility to infection with Potato yellow vein virus (PYVV). Under greenhouse conditions, the plants were inoculated with PYVV using viruliferous Trialeurodes vaporariorum (Westwood) whiteflies, and monitored for three months (the tuber formation period of S. phureja) for symptoms of infection and virus capsid protein (CP) production, by means of a reverse transcription-polymerase chain reaction (RT-PCR) assay. Symptoms of PYVV infection, consisting in the chlorotic flecking of leaflets, followed by complete vein yellowing, were noted in 38 accessions whose RNA extracts generated correctly sized CP amplicons in RT-PCR assays. Thirteen of the remaining accessions were found to be latent infected with the virus through positive RT-PCR assays. Twelve accessions were free of virus infection as judged by the absence of symptoms and negative RT-PCR assay results over two cycles of tuber setting and germination. These accessions may possess field resistance to PYVV. This work is the first one to detect and define the levels of susceptibility of Colombian S. phureja accessions to PYVV infection, and to identify the potential existence of resistance to this deleterious Andean potato virus.

Key words: Crinivirus, PYVV, Potato virus, Trialeurodes vaporariorum.

RESUMEN
La susceptibilidad de 62 accesiones de Solanum phureja ante la infección con el virus del amarillamiento de las venas de la papa (Potato yellow vein virus - PYVV) se investigó en la Colección Central Colombiana de Solanum phureja (Bogotá-Soacha). En condiciones de invernadero, las plantas se inocularon con PYVV usando mosca blanca (Trialeurodes vaporariorum [Westwood]) como vector. Posteriormente fueron monitoreadas durante tres meses, correspondientes al ciclo de tuberización de S. phureja. Se observaron los síntomas de la infección y se detectó la cápside del PYVV por transcripción reversa de la reacción en cadena de la polimerasa (RT-PCR). En 38 accesiones se observaron síntomas típicos del virus, iniciando con moteado clorótico en las hojas, seguido de amarillamiento completo de las venas. Los extractos de RNA viral generaron el tamaño correcto del producto amplificado de la proteína mayor de la cápside (CP). Trece accesiones asintomáticas presentaron el virus en estado de latencia, como se desprende de los resultados positivos de RT-PCR. En doce accesiones asintomáticas no se detectó el PYVV por RT-PCR durante dos ciclos de tuberización y germinación. Estas posiblemente son fuente de resistencia en campo a PYVV. Este trabajo detectó y definió por primera vez los niveles de susceptibilidad a PYVV en accesiones de S. phureja en Colombia; e identificó la existencia potencial de resistencia de algunas accesiones a este virus.

Palabras clave: Crinivirus, PYVV, virus papa, Trialeurodes vaporariorum.

Introduction
Sporadic outbreaks of Potato yellow vein disease (PYVD) were first observed worldwide in Antioquia, Colombia, as early as 1943 (Alba, 1950). PYVD is currently known to be caused by Potato yellow vein virus (PYVV), a tentative species in the genus Crinivirus of the family Closteroviridae (Salazar et al., 2000; Martelli et al., 2005). PYVV, whose complete genomic sequence was reported by Livieratos et al. (2004), represents the first tripartite tentative Crinivirus to be described. Yellowing of potato leaf veins with ensuing partial or total leaf chlorosis following PYVV infection can reduce tuber production in affected plants by as much as 50% in Colombia, Ecuador, Peru and Venezuela (Salazar, 2000; Salazar et al., 2000).

PYVV is semi-persistently transmitted by the whitefly Trialeurodes vaporariorum (Westwood). Vegetatively propagated potato tubers serve as a reservoir for the virus. Epidemiological studies in Rionegro (Antioquia, Colombia) have indicated that potato, tomato and various weeds (e.g. Polygonium sp.) can all act as reservoirs for PYVV (Salazar et al., 2000). As PYVD has recently become notifiable in Colombia, it is now necessary to impose quarantine

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regulations on imported potatoes. Whitefly incidence in South-America has increased dramatically since 1970. This, coupled to the unrestricted movement of potato germplasm within Andean countries, has resulted in increased PYVV incidence (Salazar et al., 2000). Reliable methods of diagnosing PYVV infection still have to be introduced on a large scale in South-America, but visual surveys suggest that the virus has possibly spread from Colombia and Ecuador to neighbouring countries, including Peru and Venezuela, probably through Solanum phureja tubers (Salazar et al., 2000).

Previous studies of PYVV molecular variation using reverse transcription polymerase chain reaction (RT-PCR) amplification assays not only found that there was low genetic diversity in the virus (Offei et al., 2004), but also that the Peruvian PYVV isolate predominated in Colombia, where it may have originated. In turn, Guzmán et al. (2006) found that potatoes can be multiply infected with several PYVV strains.

Using viruliferous insects for inoculation purposes under greenhouse and field conditions is a recommended and effective technique for screening potato accessions for resistance to insect-borne viruses (Valkonen, 1994; Solomon-Blackburn and Barker, 2001). Said technology has been used to test testing several potato clones and cultivars for susceptibility to PYVV infection following exposure to viruliferous T. vaporariorum whiteflies in the field (Pérez and Estrada, 1987). Similarly, three commercial potato varieties (Solanum tuberosum L. cvs. Diacol, Capiro and Parda Pastusa) were tested for susceptibility to PYVV infection using caged whiteflies under greenhouse conditions. In both experiments PYVV infection was assessed by visually inspecting the plants for typical leaf symptoms. However, since the virus appears to be latent in some plants (Salazar et al., 2000), such mode of assessment is not always a reliable tool.

On these grounds, the present investigation was aimed at evaluating the Central Colombian Collection of S. tuberosum Group Phureja accessions’ susceptibility to PYVV infection following the exposure of caged, healthy plants to viruliferous whiteflies, and assessing infection through its visual symptoms in combination with an RT-PCR assay for PYVV coat protein (CP) detection to confirm the presence of the virus.

Materials and methods

The present investigation was carried out with accessions from the Central Colombian Collection of Solanum phureja (Currently Solanum tuberosum, Group Phureja) (CCCSp.) supported in San Jorge farm, located in the municipality of Soacha (Cundinamarca, Colombia). The research was supported by Universidad Nacional de Colombia, on the one hand, through the Breeding and Seed Production Program of its Agronomy Faculty and through the Plant Virus Laboratory of its Biotechnology Institute; and by Cevipapa, on the other hand.

**PYVV isolate, plant maintenance and insect transmission**

Two S. phureja L. cv. Criolla Colombiana (clone 1) plants displaying vein yellowing symptoms (Fig. 1a) and known to be infected with PYVV (Guzmán et al., 2006) were collected near the towns of Cota and Subachoque (Cundinamarca, Colombia) between 2,500 and 3,000 m a.s.l. From these field plants PYVV was vector transmitted (Fig. 1d), to two separately caged, healthy clone 1 plants, which were the PYVV donors for further vector transmission to the accessions (Fig. 1b), and whose symptoms (Fig. 1c) were used as positive control throughout the research.

Sixty two accessions were clonally multiplied via sterile foam rooted shoot cuttings. The plants were potted in soil and maintained in 1 m’ screened cages inside a greenhouse.

A non-viruliferous colony of the vector (T. vaporariorum) was separately maintained on zucchini (Cucurbita pepo) plants, a non-host for PYVV. Groups of these flies were allowed to feed on the donor plants inside their cages, thus becoming viruliferous. Leaflets from the donor plants (Fig. 1c) containing 15-20 emerging whiteflies on the underside (Fig. 1d) were cut and shaken inside a cage containing the 62 healthy accession plants. There the insects were allowed to feed for a minimum period of 48 h at 18-20°C, for the plants to get infected (Salazar, 1998, 2000; Gamarra et al., 2002). The vectors were then eliminated with insecticide. Once symptoms had appeared, the plants were allowed to set tubers, which were in turn germinated to produce infected leaves. Symptom expression was recorded weekly for each accession during the four months of S. phureja development. This process was conducted in a large cage at the Faculty of Agronomy of Universidad Nacional de Colombia, Bogota campus (2,500 m a.s.l, 18-20°C).

**Transmitting PYVV to S. phureja accessions and screening for replication**

Prior to attempting PYVV transmission with viruliferous whiteflies, 115 S. phureja accessions were screened for the presence of X, Y, S and leaf roll potato viruses using a previously-described tissue printing serological assay (Guzmán et al., 2003). These plants were also assessed for infection with PYVV by RT-PCR amplification with
primers corresponding to the PYVV CP gene, following
the procedure described by Offei et al. (2004), and making
use of an extraction buffer containing sodium sulphite to
reduce potato leaf oxidation. This allowed obtaining better
concentrations of total nucleic acid in the extracts obtained
from the infected leaves (Singh et al., 2002). Those acces-
sions found to be infected with one or more of the above
mentioned viruses were not further investigated.

All of the 62 remaining (virus-free) accessions were
allowed to set tubers over a three-month life cycle (Tab.
1), after which they were collected for screening following
germination in cages inside the greenhouse. At least two
healthy plants per accession were exposed to viruliferous
whiteflies as described above, while control plants were
exposed to non-viruliferous whiteflies in separate cages.
Visual symptoms of PYVV infection, as well as time of
appearance in days post-inoculation (DPI) were noted
over the \textit{S. phureja} three-month life-cycle. PYVV presence
(absence) in the selected symptomatic (asymptomatic)
plants was tested using the RT-PCR assay as described
above.

\section*{Results}

Fifty-three of the 115 \textit{S. phureja} accessions available in
the Colombian central collection for PYVV susceptibility
screening were discarded from assessment because of prior
infection with one or a mixture of the five potato viruses
detailed above (including PYVV). Disease development
in PYVV clone 1 control plants was seen as chlorotic leaf
flecking appearing between 28 and 32 DPI, followed by
apical vein yellowing of leaflets on the top leaves (Fig. 1c).
The most severe symptoms, namely dwarfing and prema-
ture senescence, were observed in two accessions discarded
during initial screening (Am 10 and Col 13), after also hav-
ing shown well-defined chlorotic flecks followed by vein
yellowing. The tubers harvested from diseased plants over
their complete life-cycle were consistently fewer and smaller
than those gathered from healthy plants (data not shown).
When around 95\% of the tubers from PYVV infected clone
1 plants germinated, they produced infected plants with

\begin{table}
\centering
\caption{List of the accessions analyzed in this investigation.}
\begin{tabular}{lll}
\hline
Label & CCC & Origin \\
\hline
Col 2 & & \\
Col 5 & & \\
Col 8 & & \\
Col 9 & & \\
Col 10 & & \\
Col 12 & & \\
Col 13 & & \\
Col 16 & & \\
Col 19 & & \\
Col 21 & & \\
Col 22 & & \\
Col 23 & & \\
Col 26 & & \\
Col 29 & & \\
Col 32 & & \\
Col 33 & & \\
Col 36 & & \\
Col 38 & & \\
Col 39 & & \\
Col 40 & & \\
Col 42 & & \\
Col 43 & & \\
Col 46 & & \\
Col 49 & & \\
Col 57 & & \\
Col 59 & & \\
Col 61 & & \\
Col 63 & & \\
Col 67 & & \\
Col 68 & & \\
Col 70 & & \\
Phuc 2 & & Cocuy - Boyaca \\
Phuc 3 & & Cocuy - Boyaca \\
Phuc 8 & & Cocuy - Boyaca \\
Phuc 12 & & Cocuy - Boyaca \\
mam A & & Ipiales - Nariño \\
Tornilla & & Ipiales - Nariño \\
Phuc ec 1 & & Cocuy - Boyaca \\
PI 283135 & & Introduction \\
PI 225684 & & to Wisconsin \\
PI 195198 & & University Seed \\
PI 225669 & & Bank \\
PI 275110 & & \\
Am 4 & & Unknown \\
Am 10 & & Unknown \\
Am 30 & & Unknown \\
\hline
\end{tabular}
\end{table}
obvious symptoms confirmed by RT-PCR generation of the PYYV CP amplicon.

The development of PYVV infection symptoms in the 62 tested S. phureja accessions that had been kept in the experiment demonstrated that 37 of them (58.3%) were susceptible to infection; but the elicited symptoms often differed from those found in infected clone 1 plants. Accession PI 283135 stood out for showing early symptoms (10 to 15 DPI), and often dying before the end of the normal life-cycle. The symptoms observed in accessions Col 9, 10, 12, 16, 22, 23, 29, 32, 33, 36, 38, 40, 42, 43, 46, 49, 57, 61, 67, 68, 76, 81, 95, 102, 104, 109, PI 225684, Am 4, Am 30, mam A, PI 195198 and 'Tornilla' appeared after 29 to 49 DPI, and were similar to those of clone 1. Two accessions (Phuc ec 1 and Col 73) produced much later symptoms (52 to 58 DPI) with yellowing appearing in basal, lateral sprouting leaflets. RT-PCR assays confirmed that all symptomatic accessions were infected with PYVV. Not all symptomatic plants showed the same chlorosis pattern but they all produced fewer and smaller tubers when compared to healthy plants (data not shown).

Of the remaining S. phureja accessions, thirteen (21.7% of all accessions) were asymptomatic over the three-month experimental period following inoculation with viruliferous whiteflies. These accessions were: Col 2, 5, 8, 19, 21, 26, 63, 83, 107, 110 and 112, Phuc 2 and Phuc 3. However, RT-PCR amplification assays with leaflets from germinated tubers obtained from these plants produced the PYVV CP amplicon, thus confirming latent PYVV infection.

The 12 remaining S. phureja accessions (20%) showed no PYVV infection symptoms over the three-month period that followed inoculation. When tested for PYVV CP by RT-PCR assay, their leaflets were found not to have the virus. In consequence, they were allowed two tuber production-germination cycles, after which their symptomless leaflets were subjected to the RT-PCR, and found to be still virus-free. These accessions, which may contain PYVV resistance genes for future use in potato breeding programmes, were: Col 39, 59, 70, 77, 87, 90, 92 and 97, PI 225669, PI 275110, Phuc 8 and Phuc 12. All experiments were duplicated over a period of two years with essentially identical results.

Discussion

PYVV causes dramatic potato yield reductions in Andean countries, particularly in Colombia, where up to 50% losses have been reported (Salazar et al., 2000). A PYVV strain exhibiting the same sequence as the prototype Peruvian isolate predominates in Colombia (Offei et al., 2004), suggesting a common origin. Although only three different RFLP patterns for PYVV have been reported in a large Colombian sample (Guzmán et al., 2006), T. vaporariorum, the whitefly vector of PYVV, is widely dispersed in Colombia and may have determined, together with infected tubers, a larger number of PYVV variants broadly dispersed in the field. There is, thus, an urgent need to screen potato and other solanaceous germplasm banks for resistance to PYVV.

One hundred and fifteen initial accessions were serologically evaluated for PVS, PLRV, PVX and PVY viruses, which are transmitted mechanically or by aphids, but not by whiteflies. Those proving positive were rejected from the PYVV transmission study, leaving only 62 accessions for PYVV susceptibility (resistance) analysis. Included among the latter was accession Am 10, which was found to be positive for PYVV, PVS, PLRV, PVX and PVY. Its monitoring up revealed it as a highly susceptible material when compared to the rest of accessions, as it presents early chlorosis and precocious death, possibly due to the viruses' synergistic action. A group of 35 Solanum phureja 'egg yolk' clone 1 accessions were observed to have different degrees of PYVV susceptibility as seen through symptom appearance. They were, thus, of little commercial use.

The thirteen asymptomatic accessions that tested PYVV positive in the RT-PCR assay could be attributed to viral latency, as previously suggested by Salazar et al. (2000), or to infection by the vector taking place at an advanced stage in the plant's life cycle. No tuber morphology or production observation was carried out on asymptomatic PYVV positive accessions. Interestingly, on the other hand, twelve accessions expressed no vein yellowing symptoms and tested negative in the PYVV RT-PCR assay, thereby being considered as "potentially resistant to PYVV". The differential susceptibility response may have been specific for each accession; or alternatively, each vector might have individually transmitted a single viral variant, thereby explaining the different levels of symptom expression amongst accessions.

Evidence of PVYD has currently been found in 4 Colombian potato-growing departments, both in Solanum tuberosum and Solanum phureja (Franco-Lara et al., 2009). PYVV dispersion has been increasing in Andean countries since 1985, having been detected in 23 locations from four departments in Ecuador, and in 22 locations from six departments in Peru (Salazar et al., 2000; Baker et al., 2007). This means that PYVV is efficiently transmitted not only...
by contaminated tubers but also by whitefly dispersion as its natural vector. As demonstrated by Salazar et al. (2000) and by Gamarra et al. (2002), *T. vaporariorum* is the specific vector for PYVV transmission. Notwithstanding, this insect has rendered poor efficiency in the transmission of the virus to *Solanum tuberosum* (5%) under controlled temperature (20°C) and relative humidity (80%) conditions, using 10 to 30 flies per plant with 48 and 72 h periods for acquisition and infection, respectively. Higher efficiency in transmission to *S. phureja* (85%) was found in the present work under uncontrolled environmental conditions in Bogota, which were, however, similar to those found in the field.

Even though our transmission efficiency was high, it cannot be ignored that some of the 12 accessions proving negative for PYVV (20%) regarding both symptomatology and RT-PCR over two consecutive sowing cycles, may correspond to plants which had not been efficiently infected by the vector. Their resistance should thus be tested in the field. Also, it cannot be ignored that vectors have nutritional predilection for certain hosts and particular temperatures, selecting in this way viral variants for transmission, as it has been demonstrated for *Citrus tristeza virus* (CTV), which is transmitted by aphids (D’urzo et al., 2000; Velázquez-Monreal et al., 2009).

The risk for potato-growers in Andean countries has increased due to global climate change leading to increased vector presence in all temperature ranges above sea-level, due to the virus’ transmission by contaminated tubers and to the use of susceptible plants. Chávez et al. (2008) proposed a spectral diagnosis assay based on canopy reflectance measurement at several wavelengths for monitoring the virus in the field. This would allow disease patches to be identified earlier and thus become properly controlled.

This investigation is the first one to address the screening of the Colombian Central Collection of *S. phureja* accessions for their susceptibility to PYVV. Having established that around 20% of the accessions held in the collection showed some resistance to PYVV infection, further investigation of these accessions is now proposed using RT-PCR assays for PYVV CP following exposure to viruliferous whiteflies in a natural field situation, where inoculum levels are higher than those in the greenhouse trials reported here, because of increased viruliferous whitefly density. Such an inquiry would be intended to determine whether any resistance found is directly related to PYVV or to the whitefly vector. Of particular interest in this respect are the Phuc Ec *S. phureja* accessions. In effect, two of the eight screened materials of this particular type showed no symptoms of PYVV infection following inoculation, neither in the first production cycle, nor in the two subsequent generations of tubers germinated from these plants. It is also proposed to screen other native Colombian *Solanum* species and potato germplasm banks (Estrada, 2000) for PYVV resistance using greenhouse and field trials as described above. The results should be of interest for Colombian potato-growing programmes.

**Conclusions**

The majority (58.3%) of the studied accessions were found to be susceptible to infection with PYVV. The time in which the first symptoms were observed was variable, ranging from 28 to 49 dpi, according to susceptibility to virus infection. A number of accessions (21.7%) were asymptomatic but tested positive for viral presence by RT-PCR.

In twelve of the inoculated accessions (20%), the virus was not detected either by RT-PCR or symptom monitoring, thus possibly indicating resistance, which requires field testing though. Whitlefly transmission was more efficient than that reported by Gamarra et al. (2002), although transmission methodologies and host were not the same.

To date, this is the first study to evaluate susceptibility of *S. phureja* to PYVV. The results show that there might be genetic resistance to the virus in some accessions that could be used in the future for breeding programmes.

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**Cited literature**


