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

Mónica Guzmán[1, 2] and Patricia Rodríguez[1]

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 correct-sized CP amplicons in RT-PCR assays. Thirteen of the remaining accessions were found to be latently 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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[1] Plant Virus Laboratory, Biotechnology Institute (IBUN), Universidad Nacional de Colombia, Bogota (Colombia).
[2] Corresponding author. mmguzmanb@unal.edu.co

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 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^3 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 incipient infection with PYVV by RT-PCR amplification with

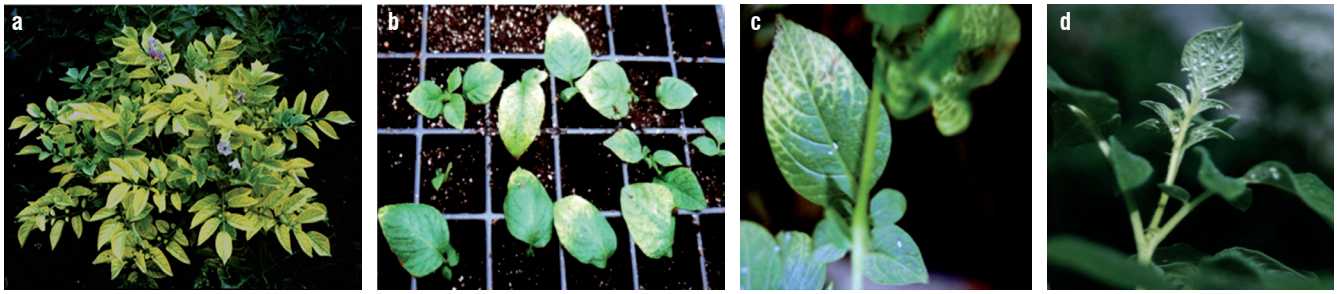

FIGURE 1. (a) *Solanum phureja* cv. Criolla Colombiana (clone 1) field plants collected near Subachoque (Cundinamarca, Colombia), displaying vein yellowing symptoms of potato yellow vein virus (PYVV) infection. (b) Plantlets of the *Solanum phureja* accessions showing yellowing symptoms after PYVV transmission. (c) Apical vein yellowing of clone 1 leaflets as seen 20 days after PYVV inoculation with viruliferous whiteflies, for further transfer of the virus to the *S. phureja* accessions in order to screen susceptibility to infection. (d) PYVV transmission by *Trialeurodes vaporariorum* whiteflies.

TABLE 1. List of the accessions analyzed in this investigation.

Label CCC	Origin	Label CCC	Origin
Col 2		Col 73	
Col 5		Col 76	
Col 8		Col 77	
Col 9		Col 81	
Col 10		Col 83	
Col 12		Col 87	
Col 13		Col 90	
Col 16		Col 92	Nariño
Col 19		Col 95	
Col 21		Col 97	
Col 22		Col 102	
Col 23		Col 104	
Col 26		Col 107	
Col 29		Col 109	
Col 32		Col 110	
Col 33	Nariño	Col 112	
Col 36		Phuc 2	Cocuy - Boyaca
Col 38		Phuc 3	Cocuy - Boyaca
Col 39		Phuc 8	Cocuy - Boyaca
Col 40		Phuc 12	Cocuy - Boyaca
Col 42		mam A	Ipiales - Nariño
Col 43		Tornilla	Ipiales - Nariño
Col 46		Phuc ec 1	Cocuy - Boyaca
Col 49		PI 283135	
Col 57		PI 225684	Introduction to Wisconsin University Seed Bank
Col 59		PI 195198	
Col 61		PI 225669	
Col 63		PI 275110	
Col 67		Am 4	Unknown
Col 68		Am 10	Unknown
Col 70		Am 30	Unknown

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 accessions 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 *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.

Results

Fifty-three of the 115 *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 premature senescence, were observed in two accessions discarded during initial screening (Am 10 and Col 13), after also having 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

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. Whitefly 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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