

Viral diseases associated to wild potatoes (*Solanum* L. section *Petota* Dumort) and its conservation in Bolivia

Mario COCA MORANTE^{1*}, Nelson TAPIA PONCE²

¹Universidad Mayor de San Simón, Facultad de Ciencias Agrícolas y Pecuarias, Departamento de Fitotecnia, Laboratorio de Fitopatología, Av. Petrolera km 5, Cochabamba, Bolivia; m.cocamorante@umss.edu.bo (* corresponding author)

²Universidad Mayor de San Simón, Agroecología Universidad Cochabamba (AGRUCO), Facultad de Ciencias Agrícolas y Pecuarias, Av. Petrolera km 5, Cochabamba, Bolivia; n.tapia@umss.edu.bo

Abstract

Different species of wild potatoes are distributed in highlands and inter-Andean valleys of Bolivia. In recent years, potato virus's incidence has been reported in native and modern varieties of Andean areas of Peru, Ecuador and Bolivia, which could also affect wild potatoes. The main of the present investigation was to identify potato viruses using DAS ELISA in wild potatoes species, from isolated collection places and intensive potato cultivation places in the Bolivian Andean region. Folioles samples from different wild potato species were collected considering isolated distribution areas and potato cultivation intensity areas. The samples were analysed using DAS ELISA for PRX, PVY, PLRV, APLV and APMoV viruses. The results show that in the high Andean zones and inter-Andean valleys some species are contaminated with PVX, PVY and PLRV viruses and not with APLV and APMoV. In the high Andean areas with intensive potato cultivation *S. acaule* is contaminated with PVX and *S. megistacrolobum* with PVY and PLRV; however, in the inter-Andean valley areas with intensive potato cultivation, *S. brevicaulis* is contaminated with PVY and *S. berthaultii* with PVY and PLRV. In isolated or remote areas *S. capsibaccatum*, *S. microdontum* and *Solanum* spp. they are not contaminated with any analysed viruses.

Keywords: disease intensity; gene losses; *in situ* conservation; plant viruses; wild genotypes

Introduction

There are about 100 wild potato species distributed from the southwestern United States to Chile, with two centers of diversity, in central Mexico and in the central Andes (Cai *et al.*, 2011). In the Bolivian Andean region, wild potatoes are distributed in high Andean areas (> 3000 m) and inter-Andean valleys (<3000 m), for example, *Solanum acaule* and *Solanum achacachense* on highland areas and *Solanum tarijense* and *Solanum berthaultii* on temperate areas (Hawkes and Hjerting, 1989; Ochoa, 1990). Crop improvement, particularly under climate change, depends on the genetic diversity of our plant genetic resources, which are arguably inadequately conserved and poorly used. Plant genetic resources are the "total genetic diversity of cultivated species and their wild relatives, much of which may be valuable to breeders" (Ford-Lloyd *et al.*, 2011). Crop wild relatives have been extremely valuable in adapting crop varieties to changing disease pressures, farming practices, market demands, and climatic conditions (Dempewolf *et al.*, 2017). According to Cai *et al.* (2011),

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extreme resistance (ER) has been identified in the wild potato species *S. chacoense*, *S. hougasii* and *S. stoloniferum*, while Hypersensitive resistance (HR) to Potato virus Y (PVY) ordinary (O) strain group (PVY^O) has been reported in *S. chacoense*, *S. demissum*, *S. megistacrolobum*, *S. polyadenium*, *S. sparsipilum* and *S. stoloniferum*.

However, some factors such as the cultivation intensity of modern varieties and climatic change to the Bolivian high Andean region could be causing some increase of virus diseases with collateral effects on wild potatoes contamination and that could affect it in the long-term conservation. Since the 1980s, surveys have been conducted using ELISA to detect the most common potato viruses (PVY, PVX, PVS, PLRV, APMoV, APLV) in potatoes growing at higher altitude (>3000 m) in the Peruvian highlands (Kreuze *et al.*, 2020). In recent years, Potato virus X (PVX), PVY, Potato leaf roll virus (PLRV) and Andean potato leaf virus (APLV) have been reported in both agro-ecological zones and the potato native crops are being affected by virus diseases (Coca-Morante *et al.*, 2020, unpublished data). The main of the present investigation was to identify potato viruses using DAS ELISA in wild potatoes species, from isolated collection places and from intensive potato cultivation places in the Bolivian Andean region.

Materials and Methods

Study area and collection

In 2017 and 2018, wild potato sample species were collected from different Andean agro-ecological zones of Bolivia (Figures 1-2). They were collected from three geographically distant points observing the Ochoa (1990) and Hawkes and Hjerting (1989) references for some species and according to our own experience references (Table 1). On the one hand, according to Ochoa (1990) and Hawkes and Hjerting references (1989), in the center of Bolivia, both the valley central and the high Andean areas of Cochabamba department, to our own references, in Pacuni locality, Sorata municipality from Larecaja Province of La Paz department, and finally, in southern Bolivia, near Tupiza city, Tupiza municipality from Province Sud Chichas of Potosí department (Figure 1) (Table 1). Taxonomy group, ploidy of each specie collected and the Agro ecological characteristics of each collection place is summarized in the Table 2.

Leaf samples and DAS ELISA virus analysis

From each selected area, leaflet samples of specimens were collected for serological analysis with DAS ELISA test. The sample collection points were random where plant populations of each species were detected. And at each point, 3 leaflets were collected per plant and between 3 to 5 plants per point. In each point were collected leaflets showing no symptoms (apparently healthy plants), mild and moderate viral symptoms (n=3 leaflet of each type (plant) per plot and 3 to 5 plots per point collection, that means 9 to 15 leaflets per specie per locality. Total n=91-115 leaflets in Aymara region per year were taken from the mid-plant area during flowering (January to February). Samples were placed in BIOREBA Universal 12 × 15 cm extraction bags, following the manufacturer's protocol, to avoid contamination. These bags were then placed in Tecnopor box containing ice and taken to the laboratory. The presence of PVY, PLRV, PVX, APLV and APMoV in leaf samples was sought using Double-Antibody Sandwich-ELISA kits from BIOREBA (these kits employ polyclonal antibodies for antigen capture and visualization [conjugated with alkaline phosphatase]), according to the manufacturer's instructions. Colorimetry was performed using a Multiscan device from Labsystem. Frequency histograms were produced in order to define cut-offs and thus allow the detection of false negatives and positives. Cut-offs were determined using the formula $mean + 3s \times 1.1$, where 'mean' refers to the mean values of negative control and 's' to its standard deviation, as recommended by the kit manufacturer. The incidence (percentage) of viral infection was determined as the number of positive samples/total number of samples × 100.

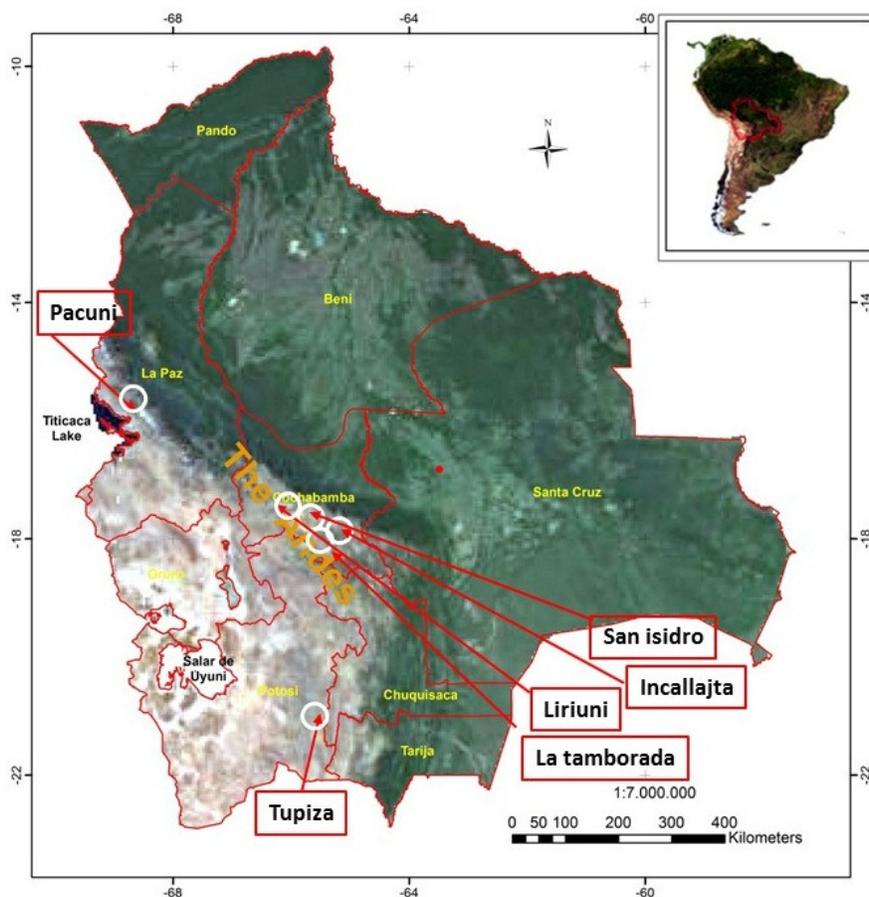


Figure 1. Map localization of the collection samples points of potato wild relatives in the Andean region of Bolivia for virus analysis

Elaboration: CISTEL - FCAYP - UMSS

Table 1. Potato wild species samples collection from different parts of the Andean region of Bolivia for viruses' identification by DAS ELISA

Locality	Province	Department	Specie	Meters	Type locality / Author
Calientes	Ayopaya	Cochabamba	<i>Solanum acaule</i>	4155	New collection area (*)
Tamborada	Cercado	Cochabamba	<i>Solanum brevicaule</i>	2570	New collection area (*)
Tupiza	Sud Chichas	Potosi	<i>Solanum brevicaule</i>	2950	New collection area (*)
Tamborada	Cercado	Cochabamba	<i>Solanum berthaultii</i>	2570	New collection area (*)
Incallajta	Pocona	Cochabamba	<i>Solanum microdontum</i>	2650	Incallajta, Hawkes and Hjerting (1989)
Liriuni	Quillacollo	Cochabamba	<i>Solanum capsicibaccatum</i>	2450	Liriuni, Ochoa (1990)
San Isidro	Sacaba	Cochabamba	<i>Solanum toralapanum</i>	3245	New collection area (*)
P'alta loma	Colomi	Cochabamba	<i>Solanum megistacrolobum</i>	3820	New collection area (*)
Pacuni	Larecaja	La Paz	<i>Solanum spp</i>	4150	New collection area (*)

(*) = Unpublished data; Years: 2017 to 2018

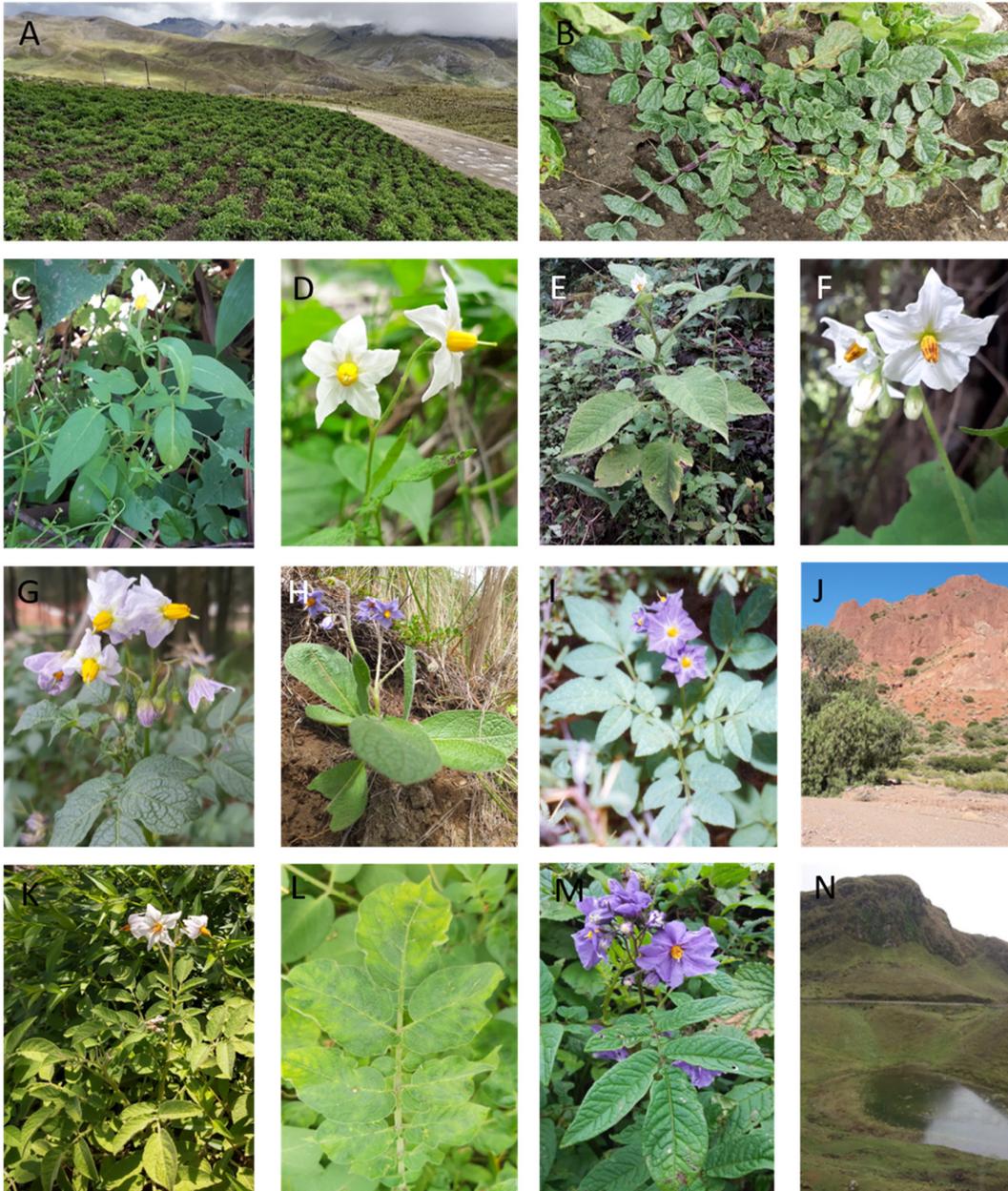


Figure 2. A: Smallholder plot whit potato luk'y variety (*S. x juzeckzukii*), Calientes locality; B: *S. acaule*, Calientes Locality; C: *S. capsibaccatum*, Liriuni, Type locality; D: *S. capsibaccatum*, stellate form; E: *S. microdontum*, Incallajta type locality; F: *S. microdontum* semi stellate form; G: *S. brevicaule*, Tamborada; H: *S. toralapanum*, San Isidro; I: *S. brevicaule*, Tupiza, Potosí; J: *S. brevicaule*, type locality, Tupiza, Potosí; K: *S. berthaultii*, type locality; L: Severe mosaic *S. ber*; M: *Solanum* spp, Pacuni, La Paz; N: *Solanum* spp., Type locality.

Table 2. Taxonomy group, ploidy of each specie collected and the Agro ecological characteristics of each collection place. Years: 2017 to 2018

Specie	Serie	Ploidy	Characteristics of sample collection place
<i>Solanum acaule</i>	Acaulia	4x	Cool and isolated high Andean area
<i>Solanum brevicaule</i>	Tuberosa	2x	Inter-Andean temperate valley of intensive agriculture
<i>Solanum brevicaule</i>	Tuberosa	2x	Temperate inter-Andean and isolated area
<i>Solanum berthaultii</i>	Commersoniana	2x	Inter-Andean temperate valley of intensive agriculture
<i>Solanum microdontum</i>	Tuberosa	2x	Temperate inter-Andean and isolated area
<i>Solanum capsicibaccatum</i>	Circaeifolia	2x	Temperate inter-Andean and isolated area
<i>Solanum toralapanum</i>	Megistacrolobai	2x	Cool and isolated high Andean area
<i>Solanum megistacrolobum</i>	Megistacrolobai	2x	Cool and isolated high Andean area
<i>Solanum spp</i>	Tuberosa	?	Cool and isolated high Andean area

Results

In 2017, wild potatoes from P'alta loma and La tamborada (Cochabamba) localities were infected with PVY-Poly and PLRV viruses, while in Pacuni (La Paz) locality they did not register any virus (Figure 3A). Therefore, there is no average virus incidence by species in Pacuni locality (Figure 2M, N), on the other hand, in *Solanum megistacrolobum* of P'alta loma locality, the incidence of PVY-Poly was 16% and PLVR of 18% and in *Solanum brevicaule* (Figure 2G) and *Solanum berthaultii* (Figure 2K, L) from the “La tamborada” locality, the incidence of PVY-Poly was 33% and PVY-Poly of 14% and PLVR was 57%, respectively (Figure 2, 3B).

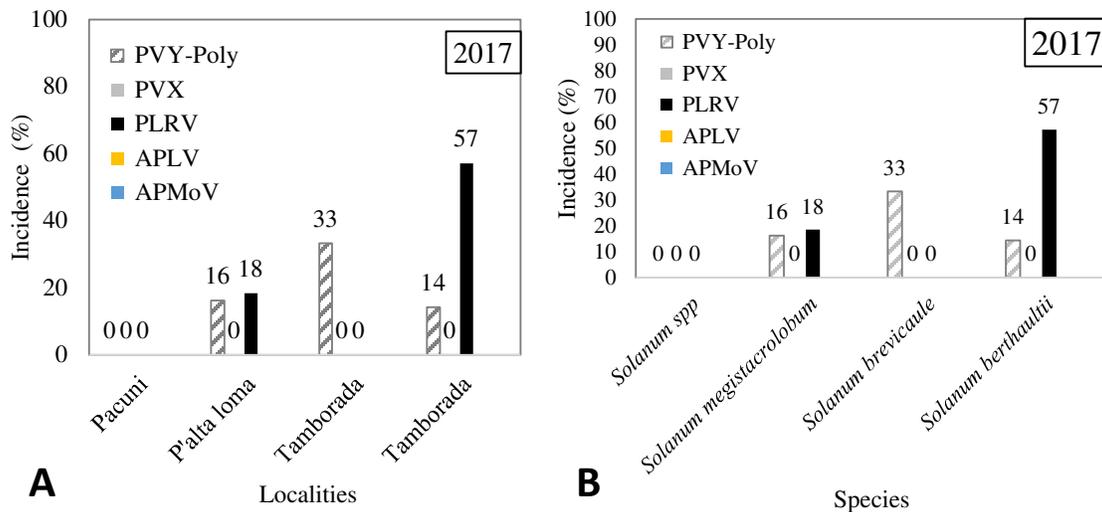


Figure 3. Viruses' incidence on potato wild relatives in the Andean region of Bolivia. A: 2017, virus incidence by locality; B: 2017, virus incidence by specie

In 2018, the wild species of the Calientes and La tamborada localities, had been infected; however, Incallajta, Liriuni, San Isidro and Tupiza localities did not register viral infections (Figure 4C). The virus incidence in *Solanum acaule* (Figure 2B) from the “Calientes” locality had PVX infection (50%) (Figure 4D), *S. brevicaule* (Figure 2M) from “La tamborada” locality showed infection with PVY-Poly (20%) and PVY-N (20%), *S. berthaultii* (Figure 2k, L) with PVY-Poly (25%) and PVY-N (25%) and *Solanum microdontum*

(Incallajta) (Figure 2E, F), *Solanum capsicibaccatum* (Liriuni) (Figure 2C), *Solanum toralapanum* (San Isidro) (Figure 2H) and *Solanum brevicaule* (Tupiza) (Figure 2I, J) did not present virus infection.

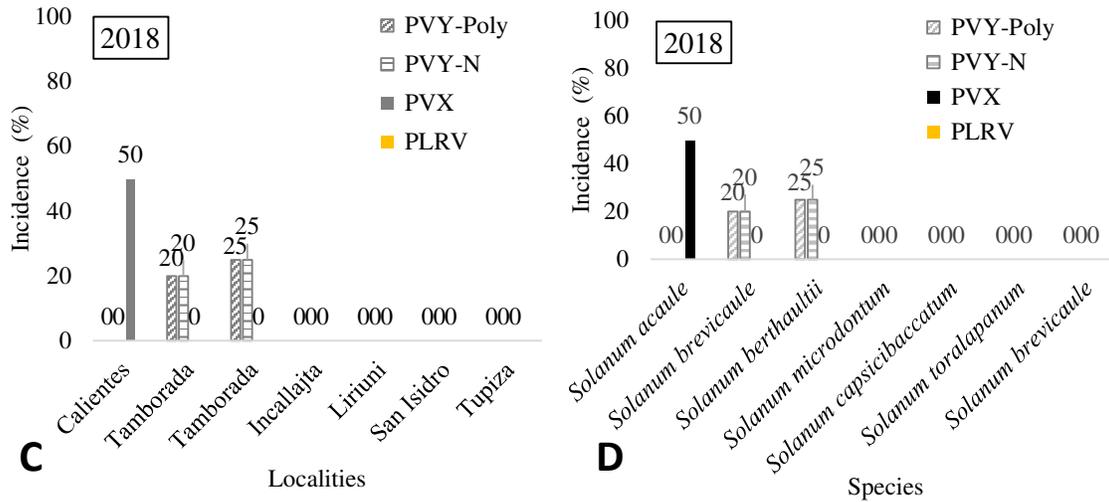


Figure 4. Viruses' incidence on potato wild relatives in the Andean region of Bolivia
C: 2018, virus incidence by locality; D: 2018, virus incidence by specie.

Discussion

The results of the present study show that wild potatoes from some sectors of the Bolivian Andean region (high Andean zones > 3,000 m and inter-Andean valleys, <3,000 m), are infected with viral potato diseases and others are not. The identified viruses were: PVX, PVY-Poly, PVY-N and PLRV, and APLV and APMoV were not registered. These identified viruses were previously reported for Andean countries, but affecting cultivated potatoes. According to Fribourg (1980), the most economically important potato viruses are PLRV and PVY, but in the highlands of the Andes, also PVX, APMV and APLV. On another hand, *S. acaule* contains resistance genes to PVX. According Bertschinger *et al.* (1990) in the Peruvian central and southern highlands, the incidence of viruses was studied in fields and tuber seeds of native and modern cultivars, and report that PVX was the most incident virus (37-82%), PVS (19-53%); PLRV and PVY were of 0.7-6.8%. It's important to note that PLRV was widely spread in native cultivars (24%). On another hand, Garcia and Gandarillas (1992), indicate that in highland area of Carrasco Province, Cochabamba department, farmers' fields planted with Huaych'a, a potato native variety, the most frequently viruses found were: PVX, APLV, APMoV and PVY, being found PVS and PLRV to a lesser extent. Later, Kreuz *et al.* (2020), say that since the 1980s, surveys have been conducted using ELISA to detect the most common potato viruses (PVY, PVX, PVS, PLRV, APMoV, APLV) in potatoes growing areas at higher altitude (>3000 m) in the Peruvian highlands and similar viruses and incidences were found in higher altitude plantings in Ecuador. Furthermore, these authors report that when similar surveys were undertaken at lower altitudes in the Andean region (<3000m), the findings resembled those in other areas of the world, with PVY and PLRV dominating.

Our results are similar to the reported by Fribourg (1980), Bertschinger *et al.* (1990), Garcia and Gandarillas (1992) and Kreuz *et al.* (2020) in relation to the PVX, PVY and PLRV importance in high Andean areas with the difference that these viruses are affecting wild potatoes as *S. acaule* (PVX), *S. megistacrolobum* (PVY and PLRV) *S. brevicaule* (PVY) and *S. berthaultii* (PVY and PLRV). These two viruses (PVY and PLRV) are consistently affecting (two years) *S. brevicaule* and *S. berthaultii*. According Fribourg (1980) and Barker and Dale (2006), *S. acaule* has resistance genes to PVX but not to PVX_{HB} and Hypersensitive Response (HR) and Extreme Resistance (ER), respectively. According Cai *et al.* (2011), *S. megistacrolobum* has HR to PVY^o

(common strain), but in our study we found that *S. megistacrolobum* is affected by PVY. According Barker and Dale (2006), two principal groups of PVY have been recognized: PVYO, or the common strain, which is severe in potato, but produces a mild mosaic in tobacco; and 2) PVYN ('necrotic' strain), which is mild in potato but is severely and systemically necrotic in tobacco, and from which the 'necrotic' name is derived. About *S. brevicaule*, *S. berthaultii* and *S. capsicibaccatum* Simko *et al.* (2007), indicate that these species are genetic resistance sources for other plant pathogens, for example, *S. brevicaule* for *Globodera pallida*, *S. berthaultii* and *S. capsicibaccatum* for *Phytophthora infestans*.

The principal modes of PVY transmission are the vegetative propagation of infected material, aphid transmission and, to a lesser extent, contact (Quenouille *et al.*, 2013; Lacomme *et al.*, 2017). By another hand Kreuz *et al.* (2020), points out that the differences in PVY and PLRV incidences between potato crops growing at different altitudes likely reflects the greater abundance of their aphid vectors below 3000 m. In a recent study on native potatoes viruses in the P'alta loma community (3750 m), Colomi, Cochabamba, the presence of different species of aphids is reported, including *Myzus persicae* (Salazar, 2020). A probable explanation in these high Andean areas is that the aphids increase could be the increase of the other diversification crops, the intensification of cultivation of modern varieties or climatic variations effects. The PVY and PLRV viruses could have an impact on long time in situ conservation and agriculture in general. According to Quenouille *et al.* (2013), the Potato Virus Y (PVY) was first associated with a disease-causing potato degeneration in the early 1930s and, Kreuze *et al.* (2020), points out that Potato Virus Y (PVY) and Potato Leaf Roll Virus (PLRV) are now the most damaging viruses of potato worldwide, with PVY having overtaken PLRV as the most important.

Conclusions

In conclusion, in the high Andean zones and inter-Andean valleys of the study area, some species of wild potatoes, are infected with PVX, PVY and PLRV viruses and not with APLV and APMoV. In the high Andean areas with intensive potato cultivation, *S. acaule*, is infected with PVX and *S. megistacrolobum* with PVY and PLRV; however, in the inter-Andean valley areas with intensive potato cultivation, *S. brevicaule* is infected with PVY and *S. berthaultii* with PVY and PLRV. In isolated or remote areas *S. capsicibaccatum* (Liriuni), *S. microdontum* (Incallajta), *S. brevicaule* (Tupiza) and *Solanum* spp. (Pacuni) they are not contaminated with any analyzed viruses.

Authors' Contributions

MCM: Study elaboration, samples collection in the field, processing laboratory, data analysis and interpretation, drafting of the manuscript, revision original draft. NTP: Project revision, supervision, data analysis and interpretation, revision of the manuscript, review and editing. Both authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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