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Scientific Research

Potyvirus Affecting Uchuva (*Physalis peruviana* L.) in Centro Agropecuario Marengo, Colombia

William Aguirre-Ráquira[1,2], Daniel Borda[3], Lilliana Hoyos-Carvajal[4]

[1]Technische Universität München (TUM), Munich, Germany
[2]Grupo de Investigación "Horticultura", Universidad Nacional de Colombia, Bogotá, Colombia
[3]Universidad Pontificia Javeriana, Bogotá, Colombia
[4]Universidad Nacional de Colombia, Medellín, Colombia
Email: wd.aguirre@tum.de, wdaguirrer@unal.edu.co

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Abstract

Fruit production and especially fresh tropical fruit trade, has an important relevance on world economy. Refining knowledge on virus diseases affecting tropical fruits is required to improve the understanding of these diseases, their dynamics and consequently, the ability to manage them. In this paper, samples of "uchuva" plants (*Physalis peruviana* L.) obtained from Centro Agropecuario Marengo (CAM) Municipality of Mosquera, Cundinamarca region of Colombia were analyzed after expressing symptoms of leaf chlorosis, leaf malformation, mosaic patterns and dwarfing. Electron microscopy revealed the presence of two different viral particles congruent with *Potyvirus* and *Tobamovirus* genus morphology. The presence of *Potyvirus* affecting the *P. peruviana* L. culture was confirmed in the samples analyzed by means of electronic microscopy images and serology. Similarly, the existence of viral particles with coherent characteristics of a putative *Tobamovirus* was observed. However, its presence could not be confirmed by means of serological tests. Nevertheless, its incidence should not be neglected. The mechanism of *Potyvirus* disease transmission in *P. peruviana* L. remains unknown, as well as the vectors associated with this disease. Therefore, complementary work and research should be considered. In addition to serology and electron microscopy, the use of indicator plants for diagnosis is suggested. Finally, a complete molecular characterization of the *Potyvirus* is recommended for a better understanding of the characteristics of its association with *P. peruviana* L.

Keywords

Plant-Virus, *Solanaceae*, Mixed-Infection, Vectors, Host-Plant, South America

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1. Introduction

Physalis peruviana L., a member of the *Solanaceae* family, is a worldwide known exotic fruit and one of the most promising fruit crops in Colombia [1], a country that actually stands with the highest production on the largest acreage of the world [2] [3]. This plant is grown as an annual or perennial culture according to the local growth conditions [4] [5]. Its fruits are characterized for being protected inside the "capacho", a common name given to the widespread calyx that facilitates its transport and also allow it to easily be sold as a fresh fruit product [6] [7]. Further potential uses include dehydrated products [8], juices, flesh pulp and sugar containing derivatives like jam [9], chocolate and ice cream [6], due to its excellent nutritional properties [10] [11]. In addition, *P. peruviana* L. is the object of diverse studies in order to take advantage of its secondary metabolites [12], which exhibit wide biological properties that could have potential pharmacological, medicinal [4] [13], and insecticidal use [10].

In Colombia, *Fusarium oxysporum* is the pathogen which causes the main problems in *P. peruviana* L. fields. [14]. At production stage, most phytopathological problems are caused by fungal pathogens like *Alternaria* spp., *Cladosporium* spp., *Phytoptora infestans* [15], *Cercospora* spp., *Phoma* spp. and bacteria as *Ralstonia solanacearum* [16] [17]. Other phytosanitary problems in *Solanaceae* are caused by several viruses. In case of *P. peruviana* L., the occurrence of the genus *Alfamovirus*, *Bigeminivirus*, *Comovirus*, *Cucumovirus*, *Fabavirus*, *Fomovirus*, *Furovirus*, *Hybrigeminivirus*, *Ilarvirus*, *Luteovirus*, *Nepovirus*, *Potexvirus*, *Potyvirus*, *Tobamovirus*, *Tospovirus*, *Tymovirus* [18]-[23] has been reported. In addition, the viroid *Potato spindle tuber viroid* (PSTVd) was reported to affect plants of *P. peruviana* L. in Turkey, New Zealand [24] and also in materials of a producer in Germany [25]. In Brazil, the presence of a *Tospovirus* was reported affecting 100% of a commercial plantation [18], which turned out to be, apparently, the first report on the occurrence in natural conditions of *Tomato Cholorotic spot virus* (TCSV). In Colombia, reports include *Cucumovirus*, *Potyvirus and Tobamovirus* genus [26]-[28] as being individually identified. In case of mixed infections, inclusion bodies similar in morphology to *Potyvirus/ Cucumovirus* genus have been reported [26]. The variation of information—though consistent—regarding the production and area sowed with *P. peruviana* L. [29], gives an important idea of the particular characteristics of Colombia's productive system and its migratory character [14]. However, as occurs with many viral diseases, it is difficult to correlate sanitary and economic information. Concretely in this case, no data were found available to associate viral diseases with economic losses in *P. peruviana* L.

This article shows evidence of the presence of *Potyvirus* associated with *P. peruviana* L. plants. The increased scientific and economic relevance of this crop worldwide imply further research and analysis on this topic in order to understand and manage virus diseases on *P. peruviana* L.

2. Materials and Methods

2.1. Biological Samples

Leaf samples from *P. peruviana* L. plants ("Colombia" ecotype) of 18 months after transplant—which showed symptoms of chlorosis, severe defoliation, mosaic and dwarfing—were collected at two different times of the year 2011 from field number five at the Centro Agropecuario Marengo (CAM) (**Figure 1**), located at 2354 m a.s.l (above sea level). During the first collection (February), samples of approximately five to eight leaves per plant were randomly collected through the field, from those expressing the symptoms above mentioned. In the case of the second collection (March), a systematic procedure was followed on plants previously selected—based on the first collection results—including only the upper and mid-section of them, with the same amount of leaves. Samples were processed by means of serological tests and electron microscopy. In order to avoid tissue damage due to natural oxidation processes all samples were preserved in cold storage (4˚C) between the different analyses, which also facilitate the description of the symptoms observed on field. The plant suspension used in the different processes was obtained only from the leaves collected and ground in the presence of phosphate buffer.

2.2. Electron Microscopy

The suspension extracted from infected leaf tissue was processed by means of negative staining using copper grids covered with the polymer "formvar". The grids were placed on the leaf extract for five minutes and later washed three times with distilled sterile water. Next, the grids were then stained in a watery solution of uranyl acetate for five minutes and finally desiccated for further manipulation under the electron microscope (JEOL JEM 1010), using the software Analysis 3.0 for the measurement procedures.

Figure 1. (a). Hyponasty (upward growth) observed in *P. peruviana* L. affected by *Potyvirus* disease. (b). Typical mosaic and hypertrophy observed on a leaf affected of *P. peruviana* L. (c). Centro Agropecuario Marengo (CAM). Km 14, vereda San José vía Mosquera-Bogotá, Cundinamarca. Exact location of the field sampled (+4˚40'57.31", −74˚12'49.08" Garmin etrex 30) (Google Maps, 2013). (d). Abnormal leaf tissue growth on symptomatic *P. peruviana* L. plants. Some leafs conserved their typical "heart shape" form even after been affected by virus diseases. (e). Fruit bearing on a virus affected plant of *P. peruviana* L. (f). Overview of the initial growing conditions of the *P. peruviana* L. field sampled.

2.3. Immunostrips

Leaves as a source of plant tissue were ground in the presence of phosphate buffer. The homogenate was transferred to 2.0 ml tubes and analyzed with immunostrips specific for *Potyvirus* and *Tobamovirus* (Kit Immunostrip Agdia®). After an incubation of 15 minutes to allow the reaction of the antigen and the antibody to take place, the immunostrips were evaluated.

2.4. PTA-ELISA

In this detection test, the antigen (sample) was covered with a sodium carbonate buffer. A specific monoclonal antibody for *Potyvirus* was used (Agdia®) and the reaction was observed at the absorbance wavelength of 405 nm (nano meter) after 30 minutes (samples first collection) and 60 minutes of incubation (samples second collection), using a ELISA Dynex-MRX reader (**Table 1**). In all tests a positive control was included for *Potyvirus* (infected leaf plant tissue), healthy leaf plant tissue was used as a negative control and sodium carbonate buffer without plant tissue was used as a blank control.

3. Results and Discussions

The obtained results confirmed the presence of *Potyvirus* associated with *P. peruviana* L. in Colombia. The images (**Figure 2(a)** and **Figure 2(b)**) showed inclusion bodies of flexuous type with a length of more than 500 nm, congruent with the characteristics of this genus [30]. Serology tests by the use of immunostrips and PTA-

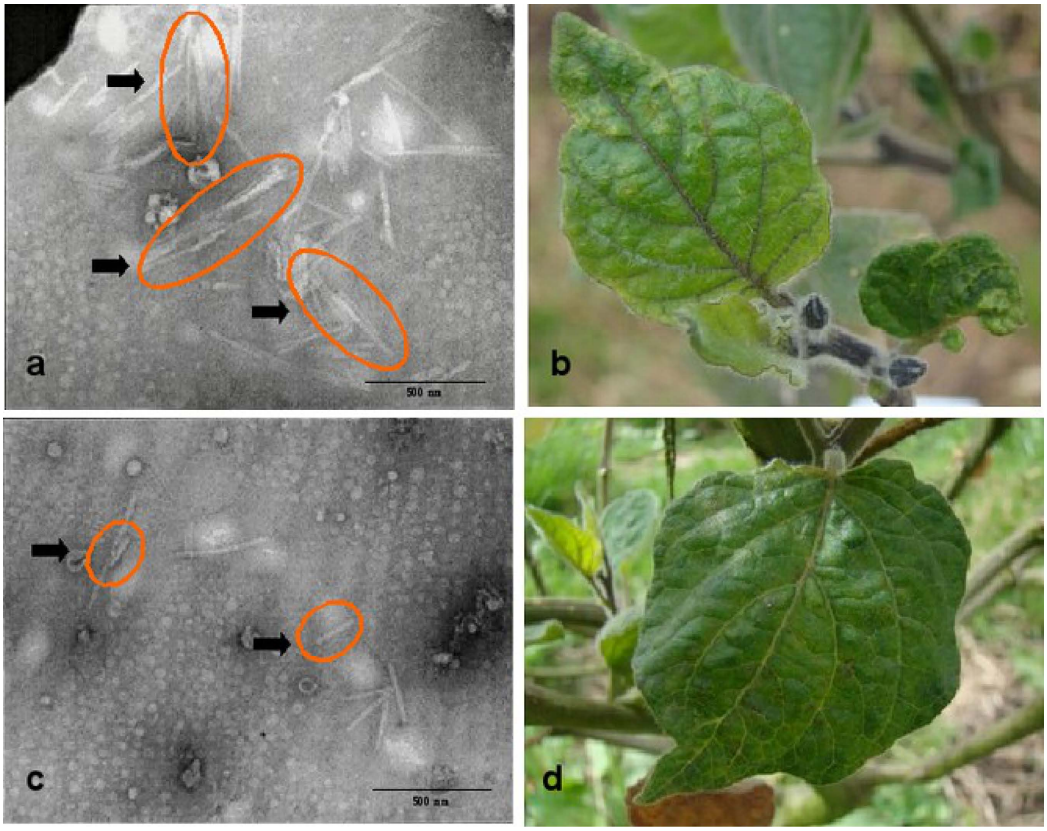

Figure 2. (a). Inclusion bodies observed and associated with the morphological characteristics of *Potyvirus* (orange ovals); (b). Chlorotic mosaic and leaf malformation as result of virus diseases on *P. peruviana* L; (c). Inclusion bodies observed and associated with the morphology of *Tobamovirus* (orange ovals); (d). Leaf malformation on *P. peruviana* L. plant infected by virus diseases.

Table 1. Results obtained by means of PTA-ELISA from samples collected, analyzed and discussed in this paper.

First collection[a]				Second collection[b]			
Sample	Label	Results		Sample	Label	Results	
		Absorbance	*Reaction*			*Absorbance*	*Reaction*
1	*P. peruviana* L. UN	0.001	(−)	1	*P. peruviana* L. B24	1.292	(+)
2	*P. peruviana* L. UN	0.003	(−)	2	*P. peruviana* L. B32	1.808	(+)
3	*P. peruviana* L. UN	0.036	(−)	3	*P. peruviana* L. C24	0.002	(−)
4	*P. peruviana* L. CAM	3.169	(+)	4	*P. peruviana* L. D20	0.012	(−)
				5	*P. peruviana* L. E28	1.641	(+)
				6	*P. peruviana* L. H02	1.790	(+)
				7	*P. peruviana* L. I23	1.683	(+)
Negative control		**0.007**	**(−)**	***Negative control***		**0.076**	**(−)**
Positive control		**3.241**	**(+)**	***Positive control***		**2.375**	**(+)**

[a]Absorbance at 405 nm after 30 minutes of reaction; [b]Absorbance at 405 nm after 60 minutes of reaction. The first collection includes random materials from the Universidad Nacional de Colombia (UN) and the Centro Agropecuario Marengo (CAM). The second collection considered only materials at CAM. In the last case the letter and number used to label the plants correspond respectively to the row and number of each of the plants sampled on field. Plus sign (+) indicates a positive reactions while minus sign (−) indicate negative reactions.

ELISA (**Table 1**) confirmed also the presence of *Potyvirus*. In addition, another viral particle (**Figure 2(c)** and **Figure 2(d)**) was observed suggesting a mix-infection affecting the plant. In this case, given the rigid characteristics and size between 250 and 300 nm [30] we presumed that it corresponds to *Tobamovirus* genus, which has been reported to affect members of the *Solanaceae* family and particularly the genus *Physalis* [23]. Nevertheless, the presence of *Tobamovirus* could not be confirmed by means of serology tests.

It is assumed that many *Potyvirus* isolates detected in different parts of the world come basically from infected materials of South American origin [31]. This region is considered to be the center of origin of several species of the *Solanaceae* family, which coexist simultaneously with wild species closely related. Therefore it is assumed that such viral diversity is a result of the co-evolution and adaptation process [32] [33].

Mixed infections are common and they impede an accurate detection based on the biological characteristics of the pathogen [34], establishing a big challenge in the diagnosis process. This is because of the synergies established with the host plant, given as a result a wide range of diverse symptoms [35]. In the case of *Tobamovirus*, despite the fact that its presence could only be assumed by means of electronic microscopy but not by serology tests, its presence should not be neglected since the high diversity of both plants and viruses in South America. Negative results of PTA-ELISA for *Tobamovirus* could be explained either to a low sensitivity of the protocol or due to a low specificity of the antibody used for the *Tobamovirus* presented in the *P. peruviana* L. samples [33]. *Tobamovirus* mobility characteristics—cell to cell process—vary and change during the development of the infection and are still not completely known [36]. In this case, the use of indicator plants is a helpful option used in the detection of viral diseases, which is usually a result of multiple methods in order to improve the diagnosis process.

Only a few reports indicate problems with viral diseases in *P. peruviana* L. cultivation. Symptoms due to virus infections can appear or disappear depending on the environmental conditions [37] or behave as conditioning agents to other diseases [38]-[40]. Therefore, virus detection is required to avoid or minimize eventual losses due to unnoticed aspects that could lead to mistakes when planning the different management strategies required for this crop [24]. A correct identification of virus diseases is needed, since it has been proven that plant susceptibility to other pathogens is higher in those affected by viral diseases [41].

Virus symptoms on plants include leaf deformations, color changes in specific patterns [42] [32], local or systemic necrosis, with changes in tissue structure which finally can result in plant death. In some cases symptoms can be absent or masked [37] although the virus is present in the plant tissue. Consequently, the problem can be associated by mistake with nutrient deficiency, physiological disorders, xenobiotic agents or entomological damages [43] [40]. Regarding all these aspects it is very difficult to correlate viral diseases with economic losses suffered by the farmers [39]. The symptoms associated with the viral disease observed on *P. peruviana* L.— commonly expressed as a chlorotic mosaic—can vary their intensity from profound (**Figure 3(a)** and **Figure 3(b)**) to mild (**Figure 3(d)** and **Figure 3(e)**) as a result of the expression of the physiological disorder of the plant. These symptoms can be observed on whole leaves or can be restricted to the primary site of infection depending on the level of resistance expressed by the host [37]. The abnormal growth of plant cells (**Figure 3(f)**) causing hypertrophy—abnormal stretching of cells—and consistent malformations (**Figure 2(b)** and **Figure 2(d)**) was observed as well. Both external and internal quality parameters of fruits decrease due to the effect of the virus disease resulting in a shortened shelf life [41]. However, this last aspect is not completely proven in the specific case of *P. peruviana* L.

Nonetheless, it is necessary to point out that diagnosis should not be limited to symptom expression [37]. In this respect, isothermal methods for nucleic acid amplification [44] [45] are available nowadays for the diagnosis of viral diseases, in replacement of the traditional PCR (Polymerase chain reaction). The advantages of these techniques are based on their versatility, a minimum of technical requirements (no thermocycler required), high specificity, high amplification rates, short reaction times [46] and the possibility of being observed with the naked eye [47]. Though at the beginning, these methods have been used in medicine [48], some works show their potential use in *Potyvirus* detection in plum trees in Germany [47].

Another aspect to be considered is the possibility of alternative host plants for the virus that affect the *P. peruviana* L. culture, among them the weeds population growing along the fields. For Cundinamarca region of Colombia, *Galinsoga* spp, *Raphanus raphanistrum* L., *Veronica persica*., *Hypochoeris radicata* L., *Holcus lantus* L., *Rumex acetocella* L., *Polygonum nepalense* M. and *Rumex crispus* L. are frequently found [49]. Special attention should be paid to the last two species mentioned, as they are known for their high frequency on *P. peruviana* L. fields in Cundinamarca region (28% and 16 % respectively). The observation of mosaic patterns and leaf

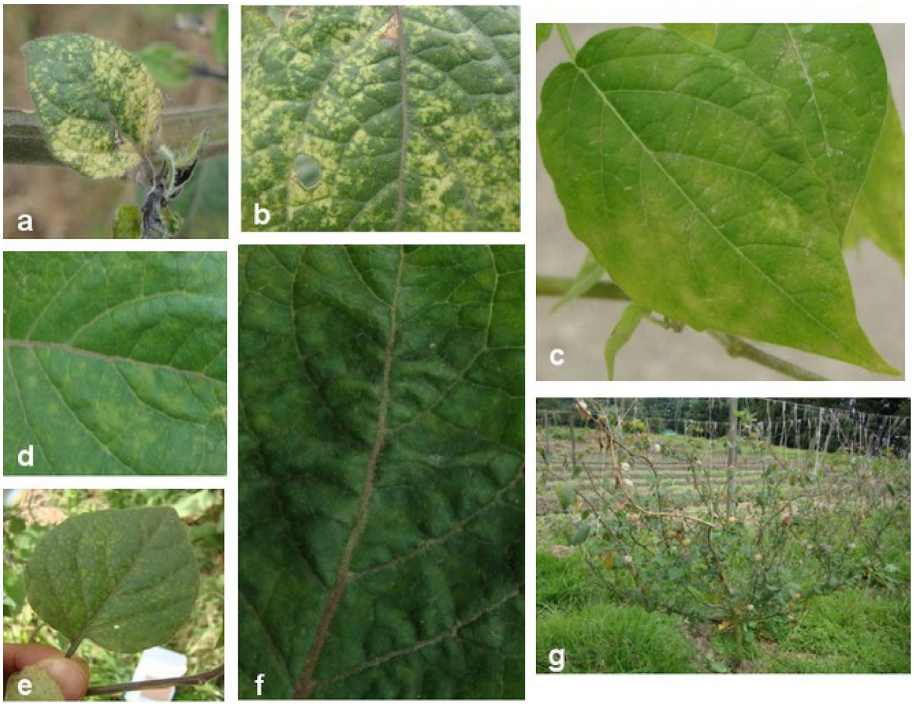

Figure 3. (a) and (b) Severe mosaic associated with symptoms generated from viral diseases in *P. peruviana L.* (c) *P. peruviana* L. used as indicator plant, expressing typical symptoms (chlorotic mosaic) caused by virus diseases; (d) and (e) Mild mosaic affecting *P. peruviana* L. (f) Typical hypertrophy observed on plants affected by viral diseases; (g) Severe defoliation on *P. peruviana* L. plant affected by viral diseases.

deformation similar to the ones observed in *P. peruviana* L. plants affected by virus (*Rumex* case) and the fact that they have been mentioned in previous reports as alternative host plants for *Potyvirus* [30] [50] supports this statement.

Within the possible virus vectors of the virus found in *P. peruviana* L., a wide range of insects and arthropods affecting different phenological states of the plant are present: *Trialeurodes vaporariorum* Westwood; *Aphis* spp., *Myzuspersicae* [26]; *Aculops lycopersici*, *Tarsonemus* spp. (Mites) [51] [52] and Trips (*Frankliniella* spp.) [53], *Lepidoptera* species of the *Noctuidae family* (*Spodoptera frugiperda* Smith and *Copitarsia decolora* Guenée) [54].

Mechanical transmission of viral diseases of *P. peruviana* L. must be considered due to the regular practices and management procedures established for this crop [14] and due to reports about this mode of transmission of several species within *Potyvirus* and *Tobamovirus* genus [19] [41] [30] [50]. Additionally, although vegetative propagation methods are not common in Colombia for *P. peruviana* L., they should be considered in the case of virus spreading due to the common pruning practices within this crop. On the other hand, seed transmissible viruses and known viroids affecting [55] *P. peruviana* L. [5] are also distributed in this way [25] [56]. In Colombia, seeds—seed selection, seedlings production and sowing of these seedlings—constitute the main method used by growers for the establishment of their *P. peruviana* L. fields [2] [14] [57]. Thus, the possibility of viral transmission by means of physical methods should not be overlooked. Furthermore, even the identification and determination of promissory materials of *P. peruviana* L. [58] must be intended by complementary plant pathology criteria to avoid the spread of these virus diseases.

4. Conclusions

The molecular characterization of *Potyvirus* associated with *P. peruviana* L. is required to improve its detection and also to distinguish it from the presence of other potential virus diseases affecting this culture, especially given the intra-specific variability that they possess. In addition to this, there is not a clear identification of the transmission mechanism of this viral disease or its possible associated vectors, and the reason for which it is necessary to coordinate works that intend to clarify these aspects.

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Dedicated to the memory of Roso Arsenio Ráquira Urián.

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