

Detection of Colombian datura virus infecting Brugmansia × candida medicinal cultivars and evaluation of sap inoculation in Solanaceae plants

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Research Article

Keywords: virus detection, leaf mosaic symptoms, leaf deformation, Potyviridae, RNA-Seq data analysis, Potyvirus trompetae

Posted Date: January 23rd, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-5845050/v1>

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Abstract

Angel's trumpets (*Brugmansia* spp.) are important medicinal plants belonging to the *Solanaceae* family that were domesticated in the Andean region of South America. Indigenous communities from the Colombian Andes have created cultivars with unusual leaf shapes and different medicinal uses. These cultivars exhibit symptoms on leaves associated with viral infection, such as mosaic, vein chlorosis, and morphological deformities. Previous studies have shown that the most widespread virus in *Brugmansia* is the *Colombian datura virus* (CDV), a globally distributed potyvirus that also affects agriculturally significant hosts. The present study aimed to evaluate the health status related to viral infections in Colombian *Brugmansia* medicinal cultivars and the relationship between the presence of viruses and the expression of symptoms in their leaves. We searched for CDV in the transcriptomes from a diverse collection of *Solanaceae* species and found it mainly in *Brugmansia* medicinal cultivars and wild solanaceous species. Sap from leaves of *B. × candida* cultivars with different symptoms were used as a source of CDV inoculum to infect representative cultivated plants of the *Solanaceae* family. We confirmed CDV infection of mechanically inoculated plants by RT-PCR and sequencing. Furthermore, we confirmed CDV ability to cause leaf deformations in agriculturally important plants such as *Physalis peruviana*, *Solanum lycopersicum*, and for the first time, reported the symptoms of the infection in *Solanum melongena*. In conclusion, this study is pioneering in characterizing a virus involved in the domestication of a medicinal plant.

Introduction

The genus *Brugmansia* is a member of the ethnobotanically important *Solanaceae* family, and contains plants used around the world for ornamental purposes, due to their showy flowers, and for medicinal purposes, owing to the presence of their tropane alkaloids, which are anticholinergic substances with a large set of applications [2, 3] and considered essential medicines by the World Health Organization. *Brugmansia* constitutes a unique example of domestication of a medicinal plant: Traditionally, indigenous communities from South America have utilized *Brugmansia* in ritualistic practices and traditional medicine, taking advantage of the psychoactive, antibiotic, anesthetic, and anti-inflammatory effects induced by the alkaloids found in their foliar tissues [2, p. 168;4]. In some places this process involved the creation of medicinal cultivars, namely clones or lineages of plants selected for specific morphological traits and medicinal attributes that are distinct, consistent, and stable [5]. The use of these cultivars is restricted to specific indigenous groups and limited geographic areas, like the south of Colombia [1]. The greatest diversity of medicinal cultivars is found in the Nariño and Putumayo Departments of Colombia. In this region, different medicinal cultivars of great ethnobotanical importance have been selected based on the medicinal uses, as well leaf shape traits like size, lamina size and symmetry [1, 4]. Recognized *B. × candida* cultivars from the Andes region hold significant importance in local communities due to their diverse applications. These plants are employed topically, in baths, or through systemic absorption, for the treatment of conditions like rheumatism, arthritis, fractures, cramps, scalds, burns, inflammations, hemorrhoids, and treatment for the protozoan skin infection leishmaniasis, among others (1, p.187; 6; 4, as cited in, 7, p.63).

Some cultivars of *Brugmansia x candida* exhibit mosaic patterns, vein chlorosis, and morphological deformities on their leaves. The cultivars 'Búyes', 'Dientes' and 'Ocre' are characterized by their ovate to elliptical leaves. However, in 'Dientes', younger leaves occasionally display distinctive deformities in their shape. While others, such as 'Amaron', 'Munchiro', and 'Quinde' have asymmetrical leaf shapes, often exhibiting deformations of the leaf blade. Lastly, 'Culebro' has been named for its long, slender leaf shape (1, pp. 179, 220). Nevertheless, leaf deformities present different levels of stability, since in cultivars with deformed leaves, vigorous shoots with regular shapes can be found. On the other hand, when malformed branches are pruned, they can regenerate with new healthy shoots; however, deformed and reduced leaves may appear later (7, p. 273).

The causes of atypical features on leaves of *Brugmansia* has been widely debated, with suggestions pointing to possible chromosomal anomalies, which are common in medicinal cultivars [1, 4] and recessive mutations (7, p.75; 4). Moreover, these characteristics have also been associated by botanists with the induction of viral symptoms (1, p.129, p.197; 4, p. 154). Thereby, the environment could influence the extent of leaf deformations and these can disappear in branches or the progeny of plants with deformed leaves. Importantly, it was discovered that the sap of the affected plant could transmit leaf deformations to asymptomatic plants when applied to them, suggesting that the observed effects have both genetic and viral causes [8, 9]. The vegetatively propagation through clonal cuttings may facilitate the dissemination of virus-infected plant material within *Brugmansia* spp. [10], such as *Colombian datura virus* (CDV), *Brugmansia suaveolens mottle virus* (BsMoV), and *Brugmansia mosaic virus* (BruMV). These are virus ssRNA (+) genome species belonging to the Potyvirus genus of the Potyviridae family reported in *Brugmansia* [10, 8, 11].

CDV is the most studied virus in *Brugmansia* spp. exhibiting characteristic foliar symptoms: mosaic patterns, rugosity, and chlorotic spots that initially appear towards the tip of the leaf and then spread across the rest of the leaf blade, along with vein yellowing, and alterations in leaf development [7, p. 272]. Elevated temperatures can exacerbate these symptoms, which may also arise in weakened, shaded branches and in poorly nourished plants even in warm environments [7, p. 273; 12]. Genetics studies have found that CDV has likely originated in the Andes of southern Colombia and was recently dispersed worldwide, affecting a range of global agricultural hosts such as *Solanum lycopersicum*, *Nicotiana tabacum*, *Cucumis sativus*, *Solanum muricatum*, *Physalis alkekengi*, and *Physalis peruviana*, among others [10, 12–14]. Its extensive host range underscores its importance in agriculture, leading to its classification as a quarantine pathogen in South Korea [15]. Interestingly, the genome of CDV shows very low variability which has been linked to anthropogenic selection in the place of origin of medicinal cultivars in the Putumayo and Nariño departments, Colombia [10].

In this article plants of *B. x candida* are the focus of our study due to their cultural and economic importance, as well as the scarcity of information regarding viral infections in medicinal cultivars in the Valley of Sibundoy, Putumayo. We used molecular techniques and sequencing confirmed the presence of *Colombian datura virus* (CDV) in these cultivars and proved by mechanical inoculation that its infection induces leaf deformations in agriculturally important hosts.

Materials and methods

Plant material. Plants used in this study were part of a living collection maintained in the green houses of the Max Planck Tandem group “Genómica Evolutiva del Metabolismo Especializado” (GEME) at the Universidad Nacional de Colombia (UNAL). Briefly, medicinal cultivars of *Brugmansia* were collected in the departments of Nariño and Putumayo in Colombia and propagated from cuttings and seeds in the greenhouse. The collection is maintained under controlled conditions (average temperature of 32 °C and relative humidity of 100 %). In this study, we selected the following medicinal cultivars of *B. × candida*: ‘Búyes’, ‘Ocre’, ‘Quinde’, ‘Amaron’, ‘Munchiro’, ‘Culebro’ and ‘Dientes’. Leaves and roots from these plants were previously characterized with RNAseq.

Detection of virus presence using transcriptomic data. We first searched for viral sequences in transcriptomic data of 130 wild and domesticated species representing the major clades in the Solanaceae family present in Colombia. These plants are part of a living collection maintained by the GEME group in greenhouses at the campus of the Universidad Nacional de Colombia sede Bogotá. Plants in this collection are derived from seeds and cuttings collected in the field. We previously performed RNAseq on leaves and roots and deposited it at the GenBank under accession code PRJNA1070281. Reads were cleaned with Trimmomatic [16] and de novo transcriptomes from the different species were constructed with the Trinity software [17].

We searched for viral sequences in the transcriptomes of *Solanaceae* species by conducting a BLASTn search [18] sequences found in the global database of plant viruses (DPVweb) [19]. We only retrieved transcriptome hits with a minimum of 90% identity to viral sequences. The results were subjected to a filtering process to select sequences with lengths exceeding the average of all found sequences (>3000 nt), which facilitated the necessary overlap for the subsequent alignment. The sequences were aligned using the ‘Codoncode’ version 2.0.1 (CodonCode Co.) and ‘MUSCLE’ [20] programs to edit alignments and identify polymorphisms between sequences.

Evolutionary relationships. Phylogenetic trees were constructed using the MEGA 11 program [21], employing the Maximum likelihood and Neighbor joining methods with a Bootstrap test of 1000 replicates. In phylogenetic analysis we describe sequences using the NCBI/GenBank accession (see table 1).

Inoculation of plants. Mechanical inoculation tests were carried out using sap extracted from symptomatic (deformed and asymmetric or with mosaic, yellowing) leaves of *B. × candida* cultivars belonging to the GEME group collection under greenhouses. It used 0.5 g of symptomatic plant material in 5 mL of phosphate buffer (10 mM, pH 7.0), following the PM 7/153 (1) protocol [22]. Inoculation was performed on test plants of the species: *Solanum lycopersicum*, *Solanum melongena*, *Physalis peruviana*, *Brugmansia sanguínea*, *Nicotiana tabacum*, *Petunia hybrida* and *Nicotiana glutinosa*. The expression of local and systemic symptoms was evaluated between 7 and 21 days post-inoculation. Infections were carried out on mature leaves, 40 days after emergence, with 3-6 repetitions for each *Brugmansia* cultivar selected as the inoculum source. Subsequently, infection assays were performed

from symptomatic indicator plants to healthy indicator plants. Negative control was constituted by plants without sap inoculation.

dsRNA and RNA Extraction. Double-stranded RNA (dsRNA) was extracted from the selected *Brugmansia* materials. To achieve this, 15 g of infected tissue was macerated and pulverized in liquid nitrogen. The extraction protocol used corresponds to that previously reported by [23,24]. Total RNA was extracted from 100 mg of tissue using the TRIzol® reagent (Invitrogen) according to the manufacturer's standard protocol. The resulting RNA pellet was then resuspended in 100 µL of DEPC-treated water. RNA was subjected to electrophoresis at 80V in a 1.5% agarose gel, using a 1000 Kb ladder. Staining was done with ethidium bromide, and visualization was performed on Bio-Rad Laboratories' ChemiDoc MP imaging system.

Molecular detection. A detection test was performed using reverse transcription (RT) and polymerase chain reaction (PCR) with degenerate primers Cl and Nlb for *Potyvirus* region [25,26], and specific primers CDV for *Colombian Datura Virus* in the Nlb/CP region [10] (see Table 2). Reverse transcription of total RNA and dsRNA was carried out using the High Capacity cDNA Reverse Transcription Kit by Applied Biosystems™. For primers Nlb2f and Nlb3R, a 35X reaction was performed: 95°C for 45s, 45°C for 45s, 72°C for 45s, and a final extension at 72°C for 5 minutes. The reaction for Clfor and Clrev was performed at 40X: 94°C for 30s, 40°C for 30s, 72°C for 1 minute, and a final extension at 72°C for 5 minutes. For CDVv and CDVvc, a 30X reaction was performed: 94°C for 30s, 55°C for 45s, 72°C for 1 minute, and a final extension at 72°C for 5 minutes. The positive control corresponded to the extraction from plants with viral RNA detected through BLAST analysis from RNA-seq, the negative control was indicator plants grown from seeds negative for viral agents, and the blank corresponded to specific primers without a sample as the reaction control. The products obtained from each amplification were performed under the same electrophoresis conditions, as previously described.

RNA extracted from *Brugmansia* cultivars and indicator plants that exhibited a positive response to viral infection was subjected to RT-PCR amplification using specific CDV primers. The purified PCR products were sequenced by Sanger, and the resulting sequences were aligned using BLAST for further analysis. *Colombian datura virus* nucleotide sequences of 17 isolates from this study were deposited in GenBank with accession numbers PQ869650 to PQ869666.

RESULTS

Detection of *Colombian datura virus* (CDV) through transcriptome data analysis in plants of the *Solanaceae* family, including the *Brugmansia* genus.

We used BLAST to search for CDV sequences in transcriptomes from *Solanaceae* species collected across Colombia. We detected CDV sequences in all *Brugmansia* medicinal cultivars ('Ocre', 'Munchiro', 'Búyes', 'Dientes', 'Culebro', 'Biangán', 'Quinde', 'Andaqui', 'Amarón') as well as *B. suaveolens*, *B. sanguinea* and other wild and cultivated solanaceous species, including wild species such as *Datura wrightii*, *Atropa belladonna*, *Browallia americana*, *Lycianthes amatitlanensis*, *Solanum catilliflorum*, *Solanum sect.*

Cyphomandra, *Jaltomata* sp. and *Solanum tuberosum* (Supplementary Fig. S1 for further details on BLAST analysis results of *Colombian Datura Virus* sequences in *Solanaceae* collection). The nucleotide identity percentages between the CDV were very high, ranging from 97.2% to 100%, the only exception was the case of *S. tuberosum*, where the identity was 80.78%.

To identify the closest relatives of CDV strains detected in our collection we compared sequences larger than 1000bp with other reference CDV genomes from different countries and found, the alignments in native cultivars matched representative global isolates (Fig. 1a). CDV sequences in the reference genome (accession NC_020072 NCBI) and in *B. candida* cv. *Munchiro* were also compared with other *Potyvirus* sequences (Fig. 1b). A closer evolutionary relationship was found between *Colombian Datura Virus* and *Tamarillo Leaf Malformation Virus* (accession KM523548.1), while greater evolutionary divergence was observed with *Celerity Latent Virus* (accession MH932227.1), the most distant genus within the *Potyviridae* family.

Symptoms associated with viral infection in *B. × candida*.

We evaluated viral symptoms in *Brugmansia* medicinal cultivars and found mosaic patterns, manifesting as areas of chlorotic pigmentation and irregular discoloration distributed along the leaf area. Roughness was observed on some leaves, along with asymmetrical morphologies, where leaf shape and size varied between cultivars (Fig. 2a, c, d, e, f, g). Figure 2 shows the leaf characteristics of *B. × candida* cultivars, some with interveinal chlorosis on the adaxial view of the leaf blade (Fig. 2a, d) (see Fig. 2).

***Colombian Datura Virus* infects solanaceous plants of agricultural interest and other hosts**

We conducted mechanical inoculation of sap extracted from *B. × candida* cv. *Munchiro* plants showing evidence of systemic infection to evaluate if CDV and unusual morphologies can be transmitted between *Solanaceae* species. We inoculated species of agricultural interest, *S. melongena*, *N. tabacum*, *P. peruviana*, and *S. lycopersicum*. The latter three species exhibited, among other symptoms: leaf blade deformation similar to the characteristics identified in *B × candida* medicinal cultivars (Fig. 2.f). Symptom expression in test *Solanaceae* plants inoculated with *Brugmansia* cv. 'Sap' for each one infected with *Colombian Datura Virus* are described below. *Solanum melongena*: The leaf blades exhibit interveinal chlorosis, which progresses to necrotic spots in the later stages of infection (Fig. 3.a). *Nicotiana tabacum*: Interveinal chlorosis begins at the base of the leaf blade and gradually progresses to a generalized mosaic and curling of the leaves at 20 days post inoculation (dpi). Chlorotic spots develop into widespread necrosis in the later stages 40dpi (Fig. 3.b). *Nicotiana glutinosa*: Chlorotic spots extend from the base to the apex of the apical leaves, with symptoms progressing to chlorotic mottling. Curling and deformities are also observed in the leaves (Fig. 3.c). *Petunia hybrida*: Chlorotic spots are present on the edges, accompanied by interveinal yellowing of the veins, a mosaic pattern (Fig. 3.d). *Physalis peruviana*: Twisting of leaves and branches, along with a generalized mosaic pattern and folding of the leaf blades (Fig. 3.e). *Solanum lycopersicum*: Chlorotic spots accompanied by vein chlorosis, mosaic patterns, and blistering of the leaves. *Brugmansia sanguinea*: The leaf blade exhibits folding and distortion at the edges, characterized by a mosaic pattern 20dpi (Fig. 3.g).

We used RT-PCR and sequencing to confirm the presence of CDV in *Brugmansia* cultivars and the inoculated plants. RT-PCR fragments corresponding to specific CDV genes were amplified from CDVv and CDVvc primers in the NIb/CP region, from infected tissue samples of various *B. × candida* cultivars and other solanaceous plants. The obtained sequences correspond to a fragment of 511 nucleotides (Fig. 4a, 4b). Additionally, amplification was achieved using degenerate primers for *Potyvirus* employing NIb and CI (Table 2). The reactions with primers NIb2F and NIb3R produced amplicons of 350 bp. Meanwhile, the primers CIFor and CIRev, designed to conserve sequences within the CI coding region, amplified a product of approximately 700 base pairs (Fig. 4c, 4d). BLAST analysis on partial sequences of PCR products of the gene CP confirmed the identity of this virus (Supplementary Fig. S2 for further details on BLAST analysis results of *Colombian Datura Virus* sequences in evaluated *Brugmansia* spp. cultivars). Amplification was specific to all *B. × candida* cultivars, including transmission to test *Solanaceae* plants; however, cultivars such as 'Ocre' and 'Amaron with regular leaf' showed less defined amplification bands (Fig. 2.c; Fig. 4.c), possibly associated with lower viral loads since they were asymptomatic materials.

DISCUSSION

Our study evaluated the evolutionary relationships of CDV isolates by two experimental strategies: i. Analysis of the transcriptomes of multiple Solanaceae species collected in Colombia showed the presence of *Colombian Datura Virus* (CDV) in *B. suaveolens* and several *B. × candida* medicinal cultivars from the Putumayo region, as well as in wild and cultivated solanaceous plants. Previous studies have experimentally confirmed CDV infection in some of these species [27]. Phylogenetic analysis showed a close evolutionary relationship between the CDV isolates in this study and those from other geographic regions. This phenomenon has been associated with the role of humans as dispersal agents of viral isolates infecting *Brugmansia* cultivars from the Andes [10]. ii. Sequences of partial regions of the nuclear inclusion protein NIb and capsid protein CP genes obtained using degenerate potyvirus primers were examined, revealing identity levels of identity of less than 75% between species known Potyviruses. Nucleotide comparisons revealed 75% nucleotide identity of *Tamarillo leaf malformation virus* (TaLMV) with our CDV, which has been previously reported [28, 29]. Phylogenetic analysis indicated that TaLMV is the closest relative to CDV, followed by five other *Potyvirus*: *Sunflower mild mosaic virus* (SMMV), *Tobacco etch virus* (TEV), *Pokeweed mosaic virus* (PkMV), *Potato virus A* (PVA), and *Tobacco vein mottling virus* (TVMV). These observations align with previous reports [30–32].

The plant material inoculated with CDV exhibited a variety of symptoms associated with the presence of a viral agent: mosaic, vein chlorosis, chlorotic spots and leaf malformations; these symptoms have been previously documented in various infected plants by *Potyvirus* [33]. Specifically blade deformations symptoms also are associated in infections by several species of *Potyviridae* family, thus *Papaya ringspot virus* (PRSV) induced severe leaf blade distortion in *Carica papaya* L. [34]. Also, symptoms generated by TaLMV in *Solanum betaceum* include rough mosaics and severe leaf blade deformations [28]. Leaf blade deformation in *Brugmansia* cultivars has been associated with chromosomal abnormalities and identified as a recessive genetic trait with variations in expression levels. A first study

showed that virus-free seedlings of *B. x candida* cv. 'Quinde' exhibited the mutant leaf shape, suggesting a genetic basis rather than a viral origin [1]. A decade later, hybridization experiments indicated that the narrow leaf trait in *B. 'Culebro'* is recessive [4]. Further study supported the genetic hypothesis of this leaf pattern, where its induction through seed irradiation [7, p. 75]. Finally, other studies dismissed the viral explanation [9, 7]. Our results show that all medicinal cultivars are infected with CDV, independently of their leaf morphology, but CDV inoculation can induce leaf deformations. These results indicate that CDV infection was probably involved in the domestication of *Brugmansia* medicinal cultivars, however CDV infection could be not the only factor associated with induction of leaf deformities. It remains to be determined why the deformities are only present in some infected plants. Additionally, more experiments are necessary to determine if CDV can be transmitted through the seeds or can cause permanent and heritable genetic effects on the infected plants.

Several hosts of CDV, primarily solanaceous plants, have been reported. In *S. lycopersicum*, CDV was detected in cultivated plants showing viral symptoms [14]. The presence and expression of symptoms were also observed in *P. peruviana*, *Solanum muricatum*, and *Mandragora officinarum* L. [13, 35]. Additionally, some studies suggest CDV infection in *Solanum tuberosum*, although natural infections have not been documented [36, 37]. Test plant assays have demonstrated the expression of symptoms consistent with those reported in *N. tabacum*, *N. glutinosa*, *P. peruviana*, *P. hybrida*, *S. lycopersicum* [10, 38, 13, 14]. This study shows for the first time, variability in symptom expression induced by CDV in *N. tabacum*, including generalized necrosis and leaf blade malformation 40 dpi. This necrotic symptom profile has been related to *BruMV* infection in *N. tabacum* [8]. Additionally, symptoms of leaf blade deformation caused by CDV have been observed in *B. demissa*, *S. lycopersicum* var. Jubileum, *N. glutinosa*, *S. scabrum*, and *S. nigrum* [13]. Mechanical inoculation in *P. peruviana* and *S. melongena*, demonstrated for the first time that CDV can infect these hosts, exhibiting leaf blade distortion and interveinal chlorosis with necrotic spots, respectively.

CDV infection induced severe symptoms of leaf deformation in *P. peruviana* and *N. tabacum* (see Figs. 3, b2 and e1). While the underlying mechanisms of *Brugmansia* leaf shape alterations and their relationship with *Potyvirus* infection remain unresolved, recent studies highlight the potential involvement of auxin signaling in leaf morphogenesis and vascular development, mediated by factors from the Auxin Response Factors (ARFs) family [39, 40]. Developmental anomalies caused by viral suppressors of RNA silencing (VSRs) have been documented in plant infected by *Potyvirus*, where the misregulation of the Auxin Response Factor 8 (ARF8) underlies the developmental abnormalities observed in transgenic *Arabidopsis thaliana* plants expressing VSRs [41]. In *Turnip mosaic virus* (TuMV) infection in *A. thaliana*, HC-Pro VSR disrupts the regulatory functions of miR167 on auxin response factors, which was associated with abnormal leaf shape development [42]. Furthermore, other potyviruses including *Tobacco etch virus* (TEV) and *Potato virus Y* (PVY), induce differential miRNA expression in host plants, leading to increased expression of miR159, miR167, miR169 and miR171 [43]; similar results were founded in Papaya infected by *Papaya ringspot potyvirus* [44]. Reduction of miR167 expression has been linked to leaf curling and wrinkling in transgenic tobacco lines [45]. This suggests how different miRNAs may affect the expression of symptoms in viral infections caused by potyviruses

by de-regulation of the auxin response pathway, a relationship that remains unclear during CDV infection in *B. × candida* cultivars and other cultivated *Solanaceae* plants.

Conclusion

This study is a pioneer in the evaluation and detection of *Colombian datura virus* in *B. × candida* medicinal cultivars, in both asymptomatic and symptomatic plants. In addition, the CDV infection in solanaceous agricultural plants such as *P. peruviana*, *S. lycopersicum*, and *S. melongena* was induced by mechanical inoculation; the expression of symptoms in trials conducted in plants of *S. melongena* is documented for the first time to our knowledge; based on transcriptome analysis, we consider that conducting controlled inoculation studies of CDV in cultivated plants of the genus *Solanum*, such as *S. tuberosum*, *S. tuberosum* group *phureja* and *S. quitoense* plants is necessary to further evaluate their susceptibility and potential responses to the virus. This study identified symptoms associated with leaf blade deformation in *P. peruviana* plants, as well as leaf deformations in *S. lycopersicum* and *N. tabacum*. These findings support the importance of investigating the mechanisms responsible for this similar phenomenon in other solanaceous plants infected by CDV, such as the *B. × candida* cultivars that exhibit distorted leaf shapes.

Declarations

Competing interests.

The authors declare that they have no conflicts of interest.

Funding.

Genómica Evolutiva del Metabolismo Especializado (GEME) project, in collaboration with the Max Planck Institute of Molecular Plant Physiology, was led by Federico Roda Fornaguera from the Max Planck Institute. This research was funded by the Convenio 566 of 2014 between Universidad Nacional de Colombia (<https://unal.edu.co/>) and Colciencias (Now Minciencias <https://minciencias.gov.co/>).

Author contributions.

All authors contributed to the conception and design of the study. Data collection and analysis were carried out by Sergio Hernández, Oscar Oliveros, Adriana Gonzalez, and Federico Roda; plant material preparation was conducted by Maria Cecilia Delgado and Sergio Hernández. Sergio Hernández and Oscar Oliveros drafted the initial version of the manuscript, and all authors provided feedback on earlier drafts. All authors read and approved the final manuscript.

Acknowledgments.

We would like to express our gratitude to Javier Sandoval-Suarez for his invaluable maintenance of the Solanaceae collection. We also extend our thanks to Gina Paola Sierra and Pablo Andres Perez for their expertise in molecular techniques and RNA extractions, which greatly contributed to this study. Additionally, we are grateful to Paula Páez for her support in the development of various assays.

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Tables

Table 1. NCBI/GenBank accessions used in phylogenetic analysis.

Potyvirus	GenBank
<i>Tamarillo leaf malformation virus</i> COL	KM523548.1
<i>Sunflower mild mosaic virus</i> ARG	JQ350738.1
<i>Tobacco etch virus</i>	M11458.1
<i>Pokeweed mosaic virus</i> USA	JQ609095.1
<i>Tobacco vein mottling virus</i> USA	X04083.1
<i>Potato A potyvirus</i> HU	AJ296311.1
<i>Potato yellow blotch virus</i> UK	JX294310.1
<i>Celery latent virus</i> ITA	MH932227.1
CDV <i>Nicotiana benthamiana</i> CN-TW	LC771070.1
CDV <i>Brugmansia suaveolens</i> KOR	MW075268.1
CDV <i>Brugmansia suaveolens</i> KOR-Taejeon-gun	OL999301.1
CDV <i>Nicotiana tabacum</i> GER-Calberlah	OQ847405.1
CDV <i>Nicotiana tabacum</i> UK	JQ801448.1
CDV <i>Nicotiana tabacum</i> USA	NC_020072.1

Abbreviations: ARG: Argentina; COL: Colombia; CN-TW: China Taiwan; HU: Hungary; ITA: Italy; KOR: Korea; UK: United Kingdom; USA: United States.

Table 2. Primers, oligonucleotide sequences, and expected sizes of PCR fragments and reference. the primer sets used in PCR reactions.

Name	Sequence	Size (pb)	Reference
NIB2F	GTITGYGTIGAYGAYTTYAAAYAA	350	Zheng et al., 2008b.
NIB3R	TCIACIACIGTIGAIGGYTGNCC		
Clfor	GGIVVIGTIGGIWSIGGIAARTCIAC	700	Ha et al., 2007.
Clrev	ACICCRTTYTCDATDATRTTIGTIGC		
CDVv	GGGAGAGCTCCTTACCTAGC	511	Chellemi et al., 2011.
CDVvc	CCATGTATGTTTGGTGACGTACC		

Figures

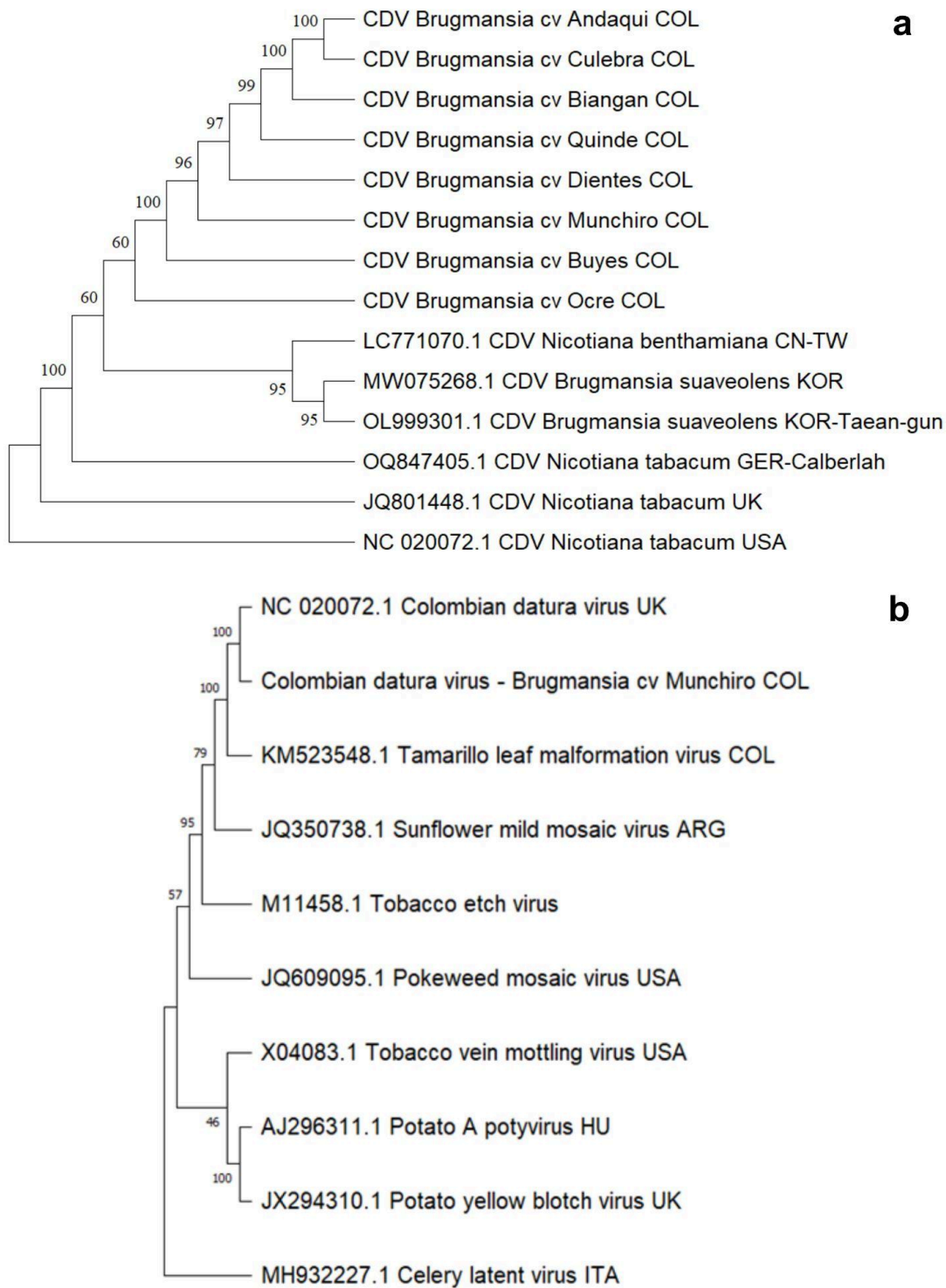


Figure 1

a. Neighbor joining Phylogenetic Tree based on deduced polypeptide sequences of *Colombian Datura Virus* (CDV) and other representative global isolates. Bootstrap analysis with 1000 replicates was performed, using accession NC_020072 as the outgroup. **b.** Neighbor joining Phylogenetic Tree of Colombian CDV isolates, the reference genome, and other *Potyvirus* species, with bootstrap analysis of

1000 replicates. *Celery latent virus* (accession MH932227.1), a member of the *Celavirus* genus within the *Potyviriidae* family, served as the outgroup.

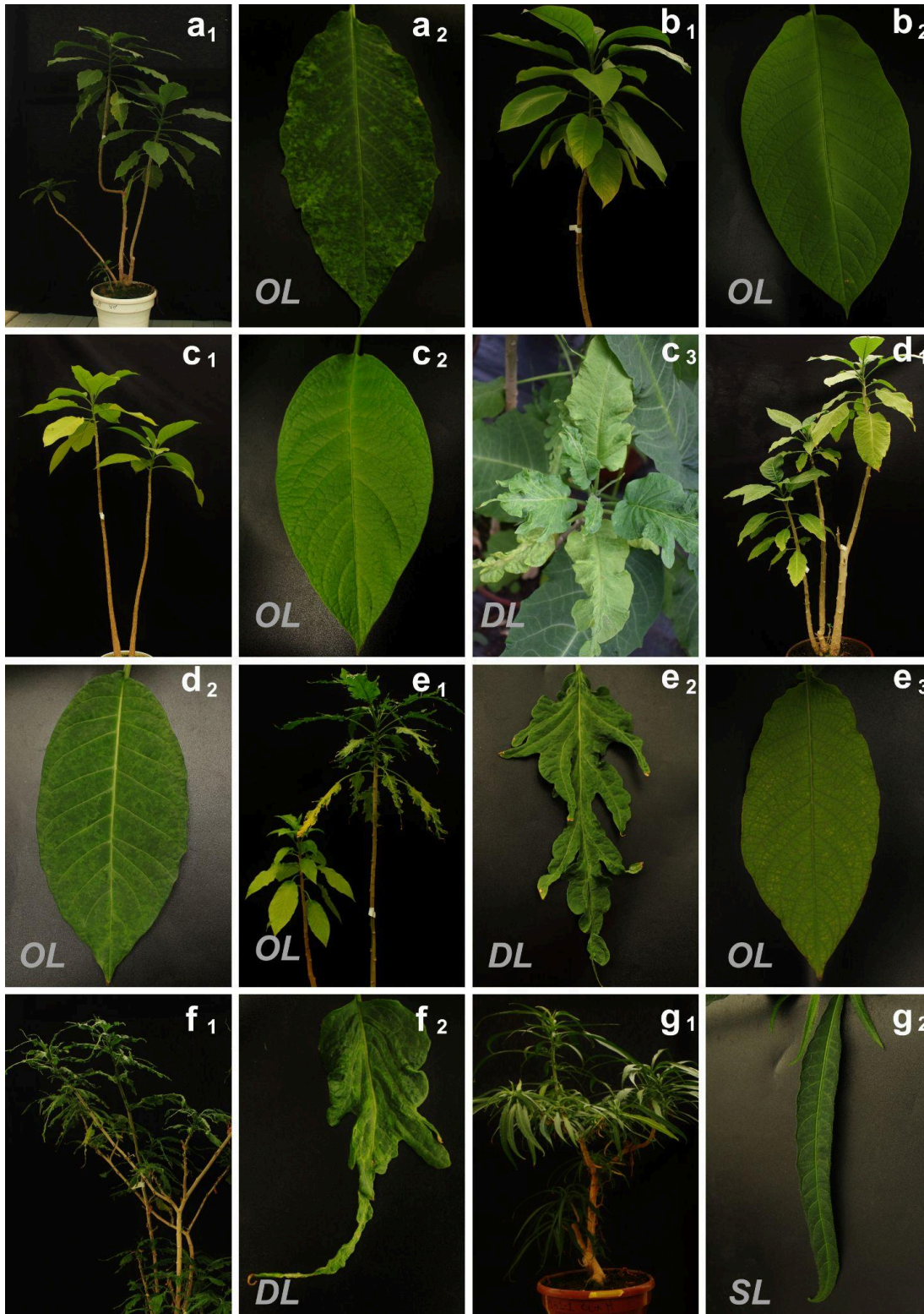


Figure 2

Symptoms on leaves of *Brugmansia* × *candida* cultivars infected by CDV. **OL**: Ovate leaf; **DL**: Deformed leaf; **SL**: Slender leaf. **(a)** 'Dientes': Mosaic and vein yellowing. **(b)** 'Ocre': No evident symptoms. **(c)** 'Amaron': Heterogeneous leaf development, including DL (deformed leaf), interveinal chlorosis, leaf blade

distortion, and apical curling; OL (asymptomatic) exhibits an ovate leaf blade. **(d)** 'Buyés': Vein yellowing and mosaic. **(e)** 'Quinde': Heterogeneous leaf development with leaf blade distortion in symptomatic material, chlorosis in main veins, apical curling, and plants with no apparent symptoms exhibiting more regular morphology. **(f)** 'Munchiro': Leaf blade distortion, vein yellowing, and apical curling. **(g)** 'Culebro': Irregularly slender leaf blade, mosaic, vein yellowing, and apical curling. **Note:** For all panels (a–g), subfigures (e.g., a1, a2) are complementary and depict different pictures of the same phenomenon.

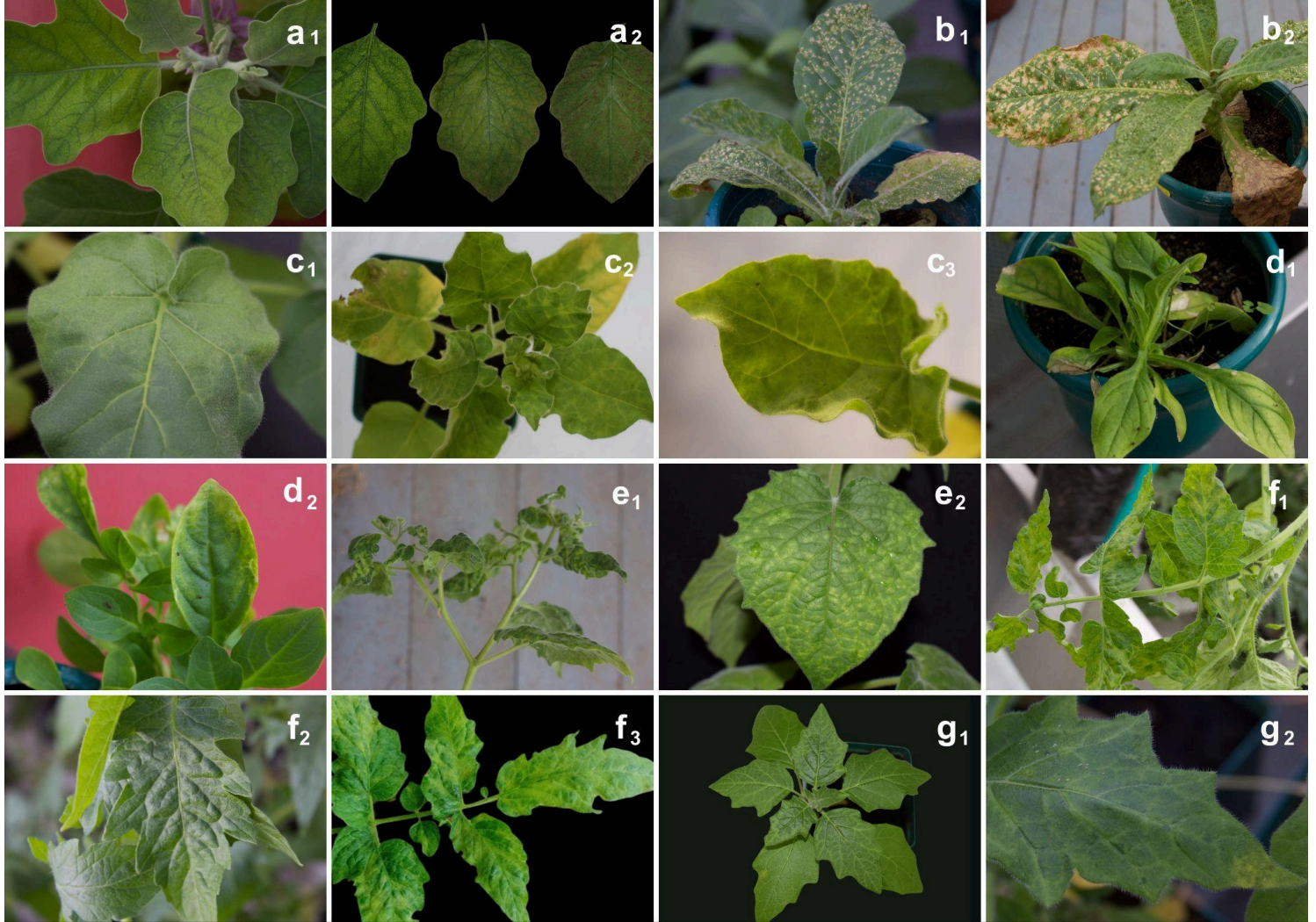


Figure 3

Symptoms observed in test plants: **(a)** *Solanum melongena*; **(b)** *Nicotiana tabacum*; **(c)** *Nicotiana glutinosa*; **(d)** *Petunia hybrida*; **(e)** *Physalis peruviana*; **(f)** *Solanum lycopersicum*; **(g)** *Brugmansia sanguinea*. (See Table 3 for description of symptoms). **Note:** For all panels (a–g), subfigures (e.g., a1, a2) are complementary and depict different pictures of the same phenomenon.

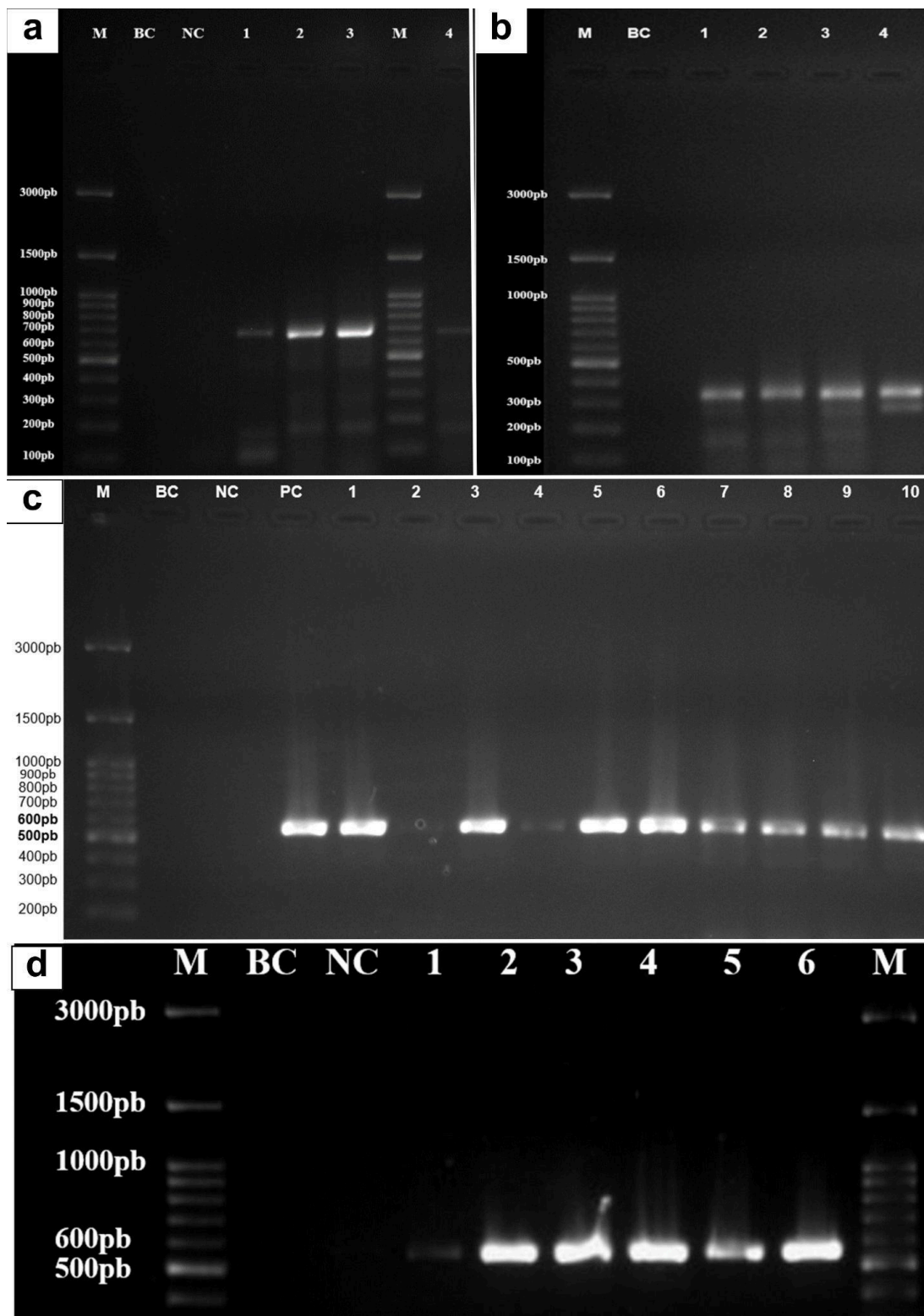


Figure 4

PCR Detection of NIb/CP potyvirus genes and *Colombian datura virus* in *B. x candida* Cultivars and Indicator Plants. **M:** Marker (100 bp + 3 kbp); **BC:** Blank; **NC:** Negative control; **PC:** Positive control; **DL:** Deformed leaf; **OL:** Ovate leaf; **SL:** Slender leaf. **(a & b)** RT-PCR fragments from degenerate primers for Potyvirus CI and NIb *region* (Ha et al., 2007; Zheng et al., 2008b) primers, respectively: 1: 'Buyés 1' (O); 2: 'Buyés 2' (OL); 3: 'Munchiro' (DL); 4: 'Dientes' (OL). **(c)** Detection of *Colombian Datura Virus* using specific

primers in the Nlb/CP region (Chellemi et al., 2011) in *Brugmansia* cultivars: 1: 'Dientes' (OL); 2: 'Ocre' (OL); 3: 'Amarón' (DL); 4: 'Amarón' (OL); 5: 'Buyés 1' (OL); 6: 'Buyés 2' (OL); 7: 'Quinde' (DL); 8: 'Quinde' (OL); 9: 'Munchiro' (DL); 10: 'Culebro' (SL). **(d)** Detection of Nlb/CP region (Chellemi et al., 2011) of CDV in indicator plants inoculated with sap from *Brugmansia* cultivars: 1: *N. tabacum*; 2: *N. glutinosa*; 3: *B. sanguinea*; 4: *S. melongena*; 5: *P. peruviana*; 6: *P. hybrida*.

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