





## Etiology, incidence, and severity of downy mildew infecting quinoa crops in Cauca, Colombia<sup>1</sup>

Luz Natalia Martínez-Caballero<sup>1</sup> , Isabel Cristina Ramirez-Paz<sup>1</sup> ,  
Kevin Alejandro Rodríguez-Arévalo<sup>1</sup> , Diana Milena Rodríguez-Mora<sup>1\*</sup> 

<sup>1</sup> Corporación Colombiana de Investigación Agropecuaria-Agrosavia, Centro de Investigación (C.I.). Palmira. Palmira (Valle del Cauca), Colombia. [lnmartinez@agrosavia.co](mailto:lnmartinez@agrosavia.co); [icramirez@agrosavia.co](mailto:icramirez@agrosavia.co); [karodriguez@agrosavia.co](mailto:karodriguez@agrosavia.co); [dmrodriguez@agrosavia.co](mailto:dmrodriguez@agrosavia.co).

\*Corresponding author: [dmrodriguez@agrosavia.co](mailto:dmrodriguez@agrosavia.co)

### Editors:

Valdir Lourenço Junior  
Danielle Fabíola Pereira da Silva

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### ABSTRACT

Downy mildew, caused by *Peronospora variabilis*, is one of the most limiting diseases affecting quinoa (*Chenopodium quinoa*) cultivation worldwide. In Colombia, there are few studies on this pathosystem. Therefore, the objective of this research was to determine the etiology, incidence, and severity of downy mildew in quinoa-producing areas in the department of Cauca. Commercial crops in the municipalities of Bolívar, La Vega, Silvia, and Totoró were visited to determine the incidence and severity of the disease, and symptomatic tissue samples were collected for studying the causal agent. The pathogen was morphologically identified using taxonomic keys and confirmed through phylogenetic analysis of the ITS and COX regions. To validate Koch's postulates, inoculations were performed using a sporangiospore suspension on healthy quinoa leaf tissue. Downy mildew was detected in all four municipalities, with incidence ranging from 3.3% to 96.7% and severity between 0.8% and 66.3%. Morphometric and molecular analyses confirmed the identity of *P. variabilis*. Pathogenicity was verified 10 days after inoculation by observing symptoms and signs on the tissue. This study presents the first confirmed report of downy mildew caused by *P. variabilis* in quinoa crops in Colombia.

**Keywords:** *Chenopodium quinoa*, COX, ITS, pathogenicity, *Peronospora variabilis*.

## INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a crop native to the Andes, renowned for its ability to adapt to various soil and climatic conditions while withstanding adverse environments.<sup>(1)</sup> Its seeds are highly nutritious, containing essential minerals, amino acids, and being considered an excellent source of protein.<sup>(2)</sup> These characteristics enable quinoa to significantly contribute to food security and sovereignty, particularly in regions facing challenges in food production.<sup>(3)</sup>

Quinoa production worldwide was estimated at 159,000 tons in 2022.<sup>(4)</sup> Bolivia, Peru, Ecuador, and the United States are the largest producers and exporters of quinoa globally; however, over 125 countries have started or plan to cultivate it as a strategy to combat malnutrition and reduce poverty.<sup>(5)</sup> In Colombia, a total of 808.3 tons were produced in 2022 across the departments of Boyacá, Cauca, Cundinamarca, and Nariño, covering an area of 453.9 hectares. Notably, Cauca accounted for 205.8 hectares and produced 227.6 tons.<sup>(6)</sup> The increase in quinoa planting area presents challenges in developing and implementing new technologies to efficiently meet the crop's needs, as well as in preventing and mitigating the phytosanitary risks associated with expanding into new territories.

The most significant disease affecting quinoa crops is downy mildew, caused by the oomycete *Peronospora variabilis* Gäum., formerly known as *Peronospora farinosa* f. sp. *chenopodii* Byford.<sup>(7)</sup> In tolerant varieties, yield losses can reach up to 35%, while in susceptible varieties, losses may exceed 90%.<sup>(8,9)</sup> The initial symptoms include small chlorotic spots on the leaves, which expand into large, irregular yellow patches. As the disease progresses, the leaves become chlorotic, curl, and premature defoliation occurs, directly impacting photosynthetic activity and reducing the plant's productive capacity.<sup>(10)</sup>

Downy mildew is widely distributed in quinoa-producing areas worldwide and has been reported in several countries, including Argentina,<sup>(11)</sup> Bolivia, Canada, Korea, Denmark, Ecuador, Egypt, the United States, India, Peru, Poland, Turkey,<sup>(12)</sup> Colombia,<sup>(13)</sup> Chile,<sup>(14)</sup> Spain,<sup>(9)</sup> Italy,<sup>(15)</sup> and Portugal.<sup>(16)</sup> However, in Colombia, information on the damage caused by downy mildew and the identity of the causal agent remains limited. Delgado *et al.*<sup>(17)</sup> evaluated the disease progression in quinoa genotypes under field conditions in the municipality of Iles (Nariño). More recently, Ramírez-Paz & Rodríguez-Mora<sup>(18)</sup> reported the presence

of the disease in quinoa crops in Silvia (Cauca). However, neither study identified the causal agent, an essential step toward understanding the pathosystem, developing effective control strategies and mitigating the disease's negative impact. Therefore, the objective of this research was to identify the pathogen associated with downy mildew in quinoa in the department of Cauca and to determine the incidence and severity of the disease.

## MATERIALS AND METHODS

### *Study area*

Between 2021 and 2023, visits were conducted to 19 commercial quinoa plots of the Blanca de Jericó genotype in the municipalities of Bolívar (5), La Vega (2), Silvia (10), and Totoró (2) in the department of Cauca, Colombia. These plots are situated at altitudes ranging from 2,171 to 2,752 meters above sea level (Table 1, Figure 1).

### *Symptoms, Incidence, and Severity*

In each commercial quinoa plot, a single field visit was carried out to identify disease symptoms and to assess the incidence and severity of downy mildew. A zigzag sampling pattern was employed to randomly select 10 plants within the cultivated area for evaluation. The symptoms associated with downy mildew were documented through photographic records taken during field evaluations.

To calculate disease incidence and severity, three leaves were randomly selected from each plant—one from each third (lower, middle, and upper)—following the methodology described by Colque-Little *et al.*<sup>(12)</sup> Incidence was calculated as the percentage of diseased leaves using the following formula:

$$\text{incidence} = \frac{\text{Number of diseased leaves}}{\text{Total leaves observed}} * 100$$

Severity was quantified as the percentage of diseased tissue using the visual scale reported by Danielsen & Ames,<sup>(19)</sup> which relates the affected leaf area from 0 to 100%. Additionally, the phenological stage of the crop was recorded based on the scale provided by Yzarra & López.<sup>(20)</sup>

### *Collection of plant material*

In the municipalities of Bolívar and Totoró, quinoa leaves of varying ages exhibiting initial and advanced

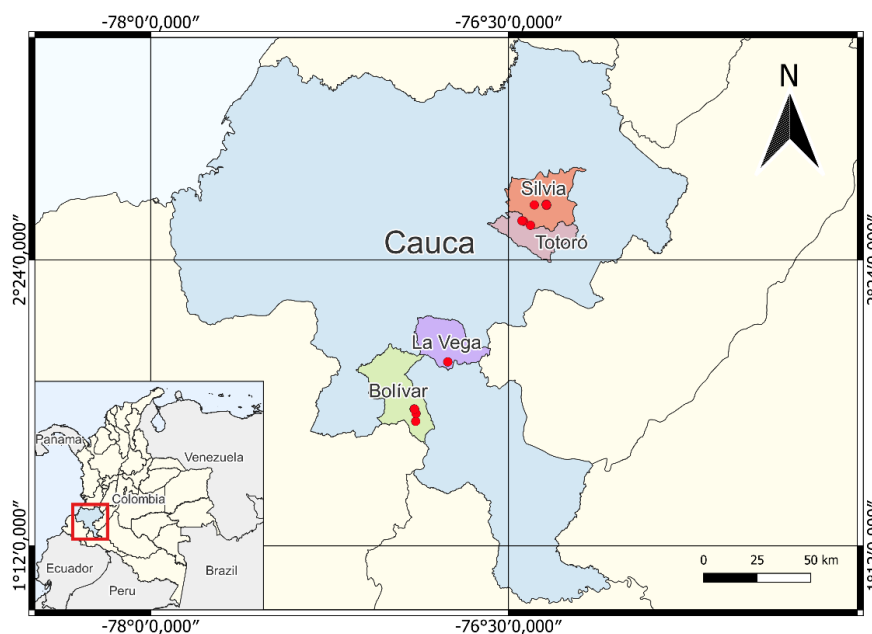
symptoms of downy mildew were collected for pathogen diagnosis. For morphological identification, two composite samples, each consisting of 10 leaves were collected from commercial fields in Bolívar (B23) and Totoró (T11). For

molecular identification, 10 samples were collected from five fields in Bolívar (B23, B24, B26, B27, B33, B34, B59, B60, B84, and B85), and four samples were collected from two fields in Totoró (T11, T12, T117, and T118) (Table 1).

**Table 1.** Incidence and severity of downy mildew in commercial quinoa plots (Blanca de Jericó) in the department of Cauca, Colombia (2021–2023)

Sample code	Evaluation date	Municipality	District	Village	Altitude (masl)	Phenological stage	Incidence (%)*	Severity (%)*
B59, B60	May-2023	Bolívar	Los Milagros	Chitacorrall	2171	Panicle development	76.7	39.5
B23, B24	May-2023	Bolívar	Los Milagros	La Zanja	2310	Flowering	56.7	15.8
B33, B34	May-2023	Bolívar	Los Milagros	La Zanja	2335	Flowering	40	7.5
B26, B27	May-2023	Bolívar	Los Milagros	La Zanja	2335	Flowering	36.7	13
B84, B85	June-2023	Bolívar	Los Milagros	El Tambo	2585	Milky grain	13.3	1.5
**	April-2022	La Vega	Pancitará	La Pila	2562	Flowering	60	22.8
—	May-2022	La Vega	Pancitará	La Pila	2728	Flowering	10	1.7
—	April_2021	Silvia	Guambia	Las Delicias	2708	Branching	36.6	11.8
—	April-2023	Silvia	Guambia	Las Delicias	2234	Branching	3.3	0.8
—	March-2021	Silvia	Guambia	Las Delicias	2706	Panicle development	61.1	20.3
—	July-2023	Silvia	Guambia	Miraflores	2501	Panicle development	10	1.7
—	April-2023	Silvia	Guambia	Las Delicias	2710	Flowering	83.3	46.5
—	August-2022	Silvia	Guambia	Las Delicias	2668	Flowering	43.3	30.5
—	July_2023	Silvia	Guambia	Tres Cruces	2752	Milky grain	96.7	25.9
—	January-2023	Silvia	Guambia	Las Delicias	2234	Milky grain	76.7	37
—	Augut_2023	Silvia	Guambia	Las Delicias	2234	Thick grain	90	66.3
—	July_2021	Silvia	Guambia	Las Delicias	2234	Thick grain	66.7	28.3
T11, T12	January-2023	Totoró	Paniquitá	La Palma	2234	Six true leaves	46.7	23
T117, T118	March-2023	Totoró	Paniquitá	La Palma	2234	Panicle development	26.7	8.4

\* Average values were calculated from 30 leaves collected from the 10 plants evaluated in each plot. \*\* Plots where no samples were collected.



Source: K. Rodríguez

**Figure 1.** Geographical location of quinoa plots marked with red spots evaluated in the department of Cauca, Colombia.

These collections were conducted between 2021 and 2023 under the framework of collection permit ANLA/1466/2014, issued on December 3, 2014, by the National Environmental Licensing Authority, available at <https://doi.org/10.15472/9zefn2>.<sup>(21)</sup> The samples were processed and preserved in the Agricultural Microbiology Laboratory of the Agrosavia C.I. Palmira (Palmira, Valle del Cauca, Colombia).

### ***Morphological identification of the pathogen***

From the initial lesions of the disease, preparations of the pathogen's reproductive structures were made on slides using sterile distilled water. Tissue segments from advanced lesions were treated with 2% KOH for 15 minutes to visualize oospores. The morphological characteristics of the pathogen were examined and measured using a light microscope (Nikon Ri2).

### ***Molecular Identification of the Pathogen***

**DNA Extraction:** Segments of tissue exhibiting early disease lesions were transferred to 50 ml polypropylene tubes and lyophilized. DNA extraction was performed using 100 mg of tissue, employing the Quick-DNA™ Plant/Seed Miniprep Kit (Catalog No. D6020) in accordance with the manufacturer's protocol.

**PCR Amplification and Sequencing:** The primers ITS1-O (5'-CGGAAGGATCATTACCAC-3')<sup>(22)</sup> and ITS4-H (5'-TCCTCCGCTTATTAATATGC-3')<sup>(23)</sup> were used to amplify a region that includes the internal transcribed spacer 1 (ITS1), the 5.8S ribosomal RNA gene, and ITS2, following the conditions reported by Göker *et al.*<sup>(23)</sup> Amplification of the mitochondrial COX2 locus, which encodes a fragment of subunit 2 (COII) of cytochrome c oxidase, was performed using the primers COX2F (5'-GGCAAATGGGTTTTCAAGATCC-3') and COX2R (5'-CCATGATTAATACCACAAATTTCACTAC-3')<sup>(24)</sup> following the conditions reported by Choi *et al.*<sup>(25)</sup> The PCR products were purified and sequenced using capillary electrophoresis with ABI 3500 equipment at the molecular genetics laboratory of the Agrosavia C.I. Tibaitatá (Mosquera, Cundinamarca, Colombia).

**Bioinformatic Analysis:** The paired reads were aligned, edited, and concatenated using Geneious Prime® 2022.2.1.<sup>(26)</sup> The processed sequences were deposited in GenBank at the National Center for Biotechnology Information (NCBI) under accession numbers PQ350322-PQ350335 (ITS) and PQ351605-PQ351618 (COX2).

Subsequently, sequences were individually submitted to BLASTn. Sequences of *P. variabilis* from different geographic origins were retrieved from GenBank, along with those of *P. boni-henrici*, *P. cheonopodii-ambrosioides*, and *P. cheonopodii-ficifolii*, which infect other species of the genus *Chenopodium*. Additionally, *P. rumicis*, a pathogen that infects *Rumex acetosella*, was included (Supplementary Table 1).

The sequences were aligned using the Muscle v3.8.1551 program,<sup>(27)</sup> and the best substitution model for each partition was selected using ModelTest-NG v0.1.7 software, based on the Bayesian information criterion (BIC).<sup>(28)</sup> The phylogenetic tree was constructed using the maximum likelihood method with IQ-TREE 2 v2.3.4.<sup>(29)</sup> The ultrafast bootstrapping method was employed to assess the support of inferred tree branches, with 1000 UFBoot replicates. Visualization was carried out with the ggtree package<sup>(30)</sup> in R.<sup>(31)</sup>

### ***Pathogenicity tests***

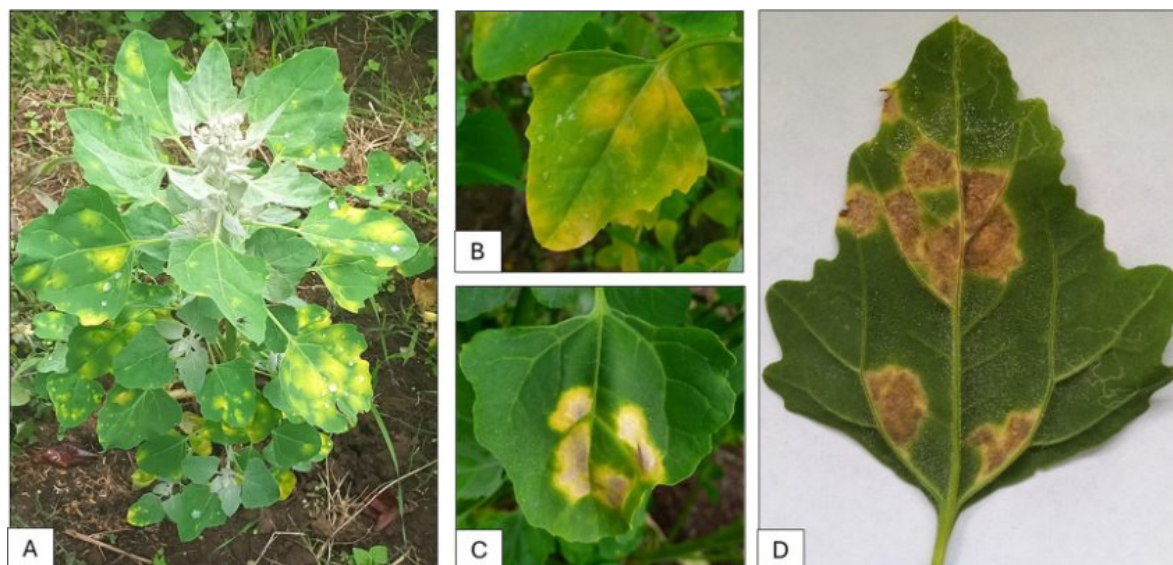
Quinoa leaves from samples B23 and T11, collected from Bolívar and Totoró, respectively, were selected. Early disease lesions from these samples were chosen, and the pathogen's reproductive structures were extracted using the methodology described by Danielsen & Ames.<sup>(19)</sup> Quinoa leaves were inoculated with a sporangiospore suspension of 4 x 10<sup>5</sup> spores/mL, applied to the adaxial side on 1% agar.<sup>(19)</sup> The incubation was conducted in a growth chamber (Binder KBWF 720) at 10 °C for 24 hours in the dark, followed by an additional 10 days at 20 °C under a 12-hour light/12-hour dark cycle with 80% relative humidity. The experimental setup was designed as a completely randomized design, with each experimental unit consisting of one leaf and 9 repetitions; leaves inoculated with sterile distilled water served as negative controls. Symptoms were monitored for 12 days following inoculation.

## **RESULTS**

### ***Symptoms***

In Blanca de Jericó, symptoms of the disease were evident on the lower, middle, and upper parts of the plant (Figure 2A). The initial symptoms were characterized by small, irregular chlorotic spots on the adaxial side of the leaves (Figure 2B). In advanced lesions, large chlorotic spots and necrotic areas were observed (Figure 2C). Additionally, a gray, cottony layer was identified on the abaxial surface of the leaf, corresponding to the reproductive structures of the pathogen (Figure 2D).





Source: I. Ramirez.

**Figure 2.** Symptoms of downy mildew in quinoa, Blanca de Jericó. A. Symptoms of the disease on the lower, middle, and upper thirds of the plant. B. Irregular chlorotic spots. C. Necrotic lesions. D. Signs of the pathogen on the abaxial side of the leaf.

### *Incidence and Severity*

Downy mildew was detected in all 19 commercial quinoa plots at various phenological stages. In Silvia, the plots were found in panicle development, flowering, milky grain, and soft dough stages, with incidence ranging from 3.3% to 96.7% and severity between 0.8% and 66.3% of the leaf tissue area affected by the disease. In Bolívar, downy mildew was recorded during flowering, panicle development, and milky grain stages, with incidence values between 13.3% and 76.7%, and severity ranging from 1.5% to 39.5%. In La Vega, it was observed during flowering stage, with incidence ranging from 10% to 60% and severity from 1.7% to 22.8%. In Totoró, the disease was recorded at the six true leaves and panicle development stages, with incidences ranging from 26.2% to 46.7% and severity between 8.4% and 23% (Table 1).

### *Morphological identification*

Sporangiophores straight to slightly curved in shape, colorless, exhibiting dichotomous branching in 2 to 5 orders, and openings at acute angles ( $n = 60$ ) were observed with a total length ranging from 181.2 to 491.3  $\mu\text{m}$ . Their base width varied from 10.1 to 21.9  $\mu\text{m}$ , while the mid-width ranged from 8.1 to 16.6  $\mu\text{m}$ . Trunk length ( $n = 70$ ) measured between 117.4 and 362.0  $\mu\text{m}$  (Figure 3A-B). The terminal branches pointed to slightly curved measured

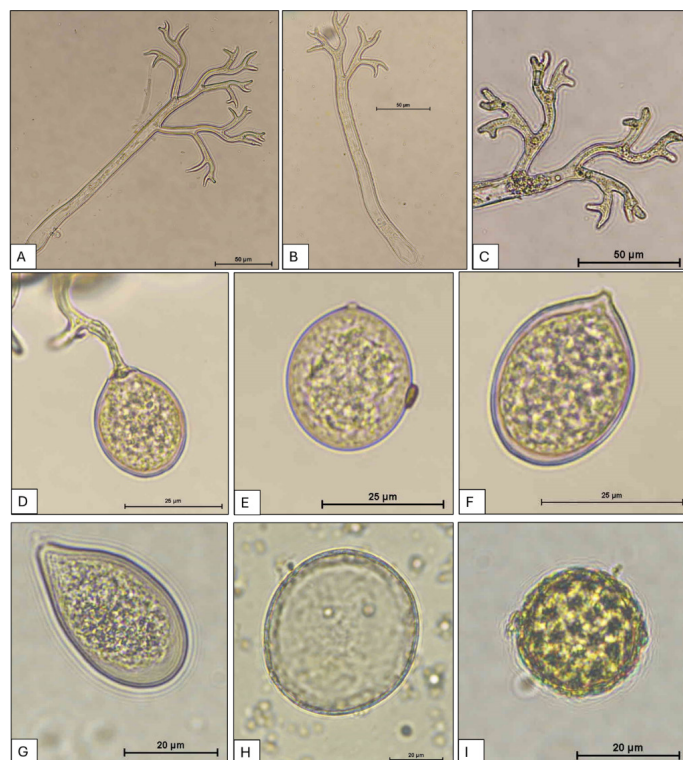
between 9.1 and 24.7  $\mu\text{m}$  in axial length and 8.1 to 15.3  $\mu\text{m}$  in abaxial length (Figure 3C).

Sporangia ( $n = 195$ ) subglobose to elliptical, oval, and rarely napiform, displaying an olive coloration, were either caducous or adhered to the terminal branches of the sporangiophores. Measured from 20.1 to 33.3  $\mu\text{m}$  in width and 26.6 to 38.7  $\mu\text{m}$  in length, with an average length-to-width ratio of 1.3 (Figure 3D-G).

Resistance structures were observed in tissue with advanced lesions of the disease. Immature oospores ( $n = 70$ ) were slightly oval and light brown, measuring from 41.2 and 72.6  $\mu\text{m}$  in width and 43.7 to 86.6  $\mu\text{m}$  in length, with a length-to-width ratio of 1.1 (Figure 3H). Mature oospores ( $n = 59$ ) were yellow to brown, rough-walled, rounded to slightly ovoid shape, measuring from 17.7 to 37.8  $\mu\text{m}$  in length and 17.7 to 36.8  $\mu\text{m}$  in width (Figure 3I).

### *Molecular identification*

Local alignments of internal transcribed spacers (ITS) region against the NCBI nt database for all 14 isolates yielded the highest bitscores corresponding to *P. variabilis* accessions. Identity percentages exceeded 99% with accessions KF269538.1, EU113303.1, MK394004.1, ON046667.1, and FM863719.2. Similarly, for the cytochrome c oxidase subunit 2 region, all the samples exhibited the highest bitscore against the *P. variabilis* accession KF269650.1, with 100% identity.



Source: L-N Martínez.

**Figure 3.** Morphological structures of *Peronospora* sp. on *Chenopodium quinoa*. A-B. Dichotomous sporangiophores. C. Branches. D. Forming sporangium attached to branches. E-G. Sporangia. H. Immature oospore. I. Mature oospore.

### Phylogenetic analysis

The sequences alignment consisted of 1,310 positions, with the first partition being the ITS region, which contained 801 positions, while the second partition, COX2, had 509 positions. The base substitution models that best fit the data were TPM2u+G4 for ITS and HKY+I+G4 for COX2.

The maximum likelihood tree revealed that 14 downy mildew samples from Bolívar and Totoró, Cauca, Colombia clustered together with reported sequences of *P. variabilis*. Two distinct clades were observed: the first clade included downy mildew sequences from Bolivia, Colombia, and Ecuador, along with sample RS from the USA. The second clade grouped samples from Spain and USA (Figure 4).

### Pathogenicity tests

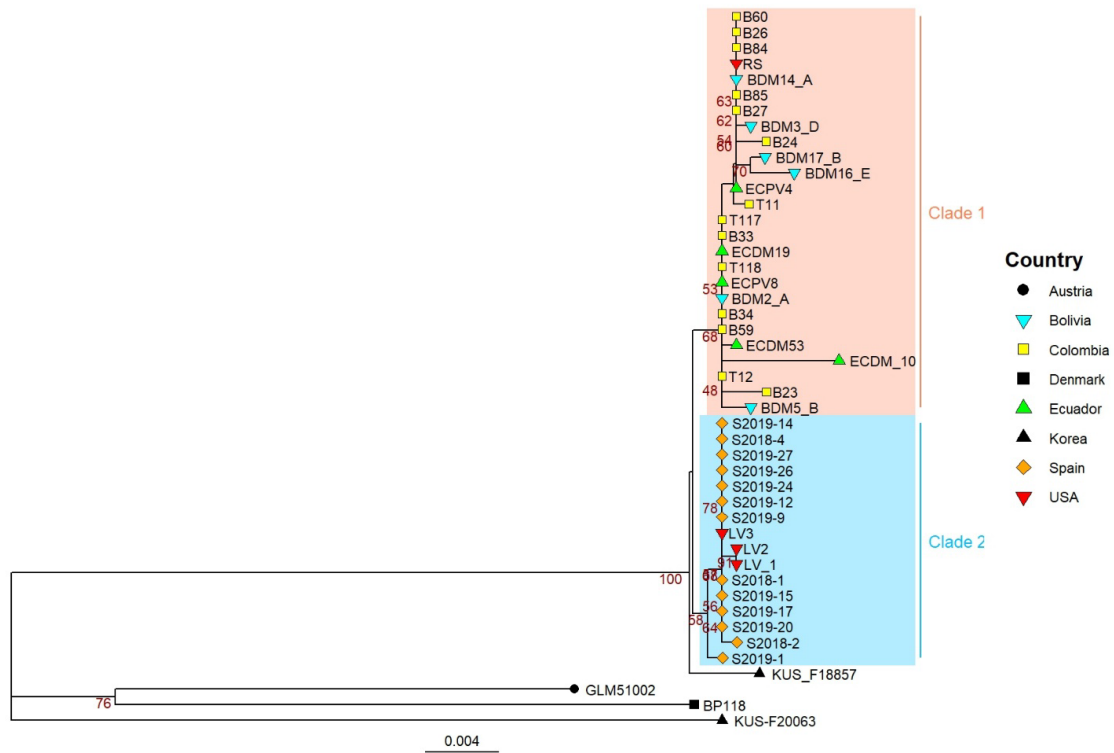
The first symptoms of the disease were observed seven days post-inoculation (dpi), with the signs of the pathogen appearing by day ten. The infection percentage was 66.6% for isolate B23 and 55.5% for isolate T11. Symptoms manifested as irregularly shaped chlorotic lesions, resembling those observed in the field (Figure 5A). Microscopic examination revealed immature sporangia and fully developed

sporangiospores of *P. variabilis* within the chlorotic lesions (Figure 5C and D). The negative control exhibited no signs of disease (Figure 5B).

### DISCUSSION

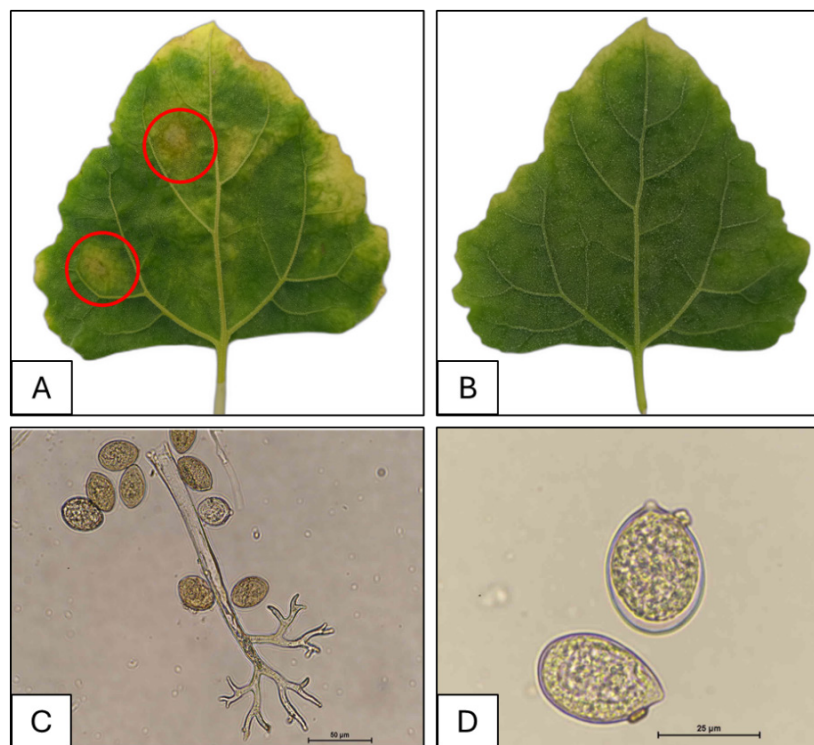
The most characteristic symptoms of downy mildew observed in Blanca de Jericó genotype were consistent across all four municipalities, showing irregular chlorotic spots on the adaxial side of the leaves and pathogen signs on the abaxial surface. These symptoms correspond with those reported by Plata *et al.*<sup>(32)</sup> and Beccari *et al.*<sup>(15)</sup> in other quinoa genotypes. As noted by Colque-Little *et al.*,<sup>(33)</sup> the disease's symptoms vary among genotypes, growth stage, and environmental conditions. In this study, downy mildew was observed across all phenological stages in the registered commercial plots, ranging from the six true leaf stage to the thick grain stage. Similar findings were reported by Danielsen & Ames,<sup>(19)</sup> who recorded the presence of the disease throughout the entire phenological cycle of the crop, including the plant emergence stage.

The early development of symptoms is attributed to pathogen oospores, which persist in various seed structures, as well as in cotyledons and radicle pith of the



Source: K. Rodríguez.

**Figure 4.** Maximum likelihood (ML) tree constructed from sequences of the internal transcribed spacer (ITS) and cytochrome c oxidase subunit 2 (COX2) obtained from downy mildew samples.



Source: I. Ramirez and L-N Martínez.

**Figure 5.** Pathogenicity test of *P. variabilis* on Blanca de Jericó quinoa leaves. A. Symptoms of infection at 10 dpi (red circle). B. Negative control. C. Sporangiophores (40x). D. Sporangium (100x).



emerging plant. Mycelium spreads from the radicle to the aerial branches, flowers, and developing seeds. Moreover, oospores found in petioles and leaf lesions can remain in soil after leaf fall, facilitating infection in the subsequent crop cycle.<sup>(34)</sup>

Based on the severity data recorded in the evaluated plots, the Blanca de Jericó genotype exhibited moderate susceptibility to downy mildew. These results align with those presented by Delgado *et al.*<sup>(17)</sup> who reported that this genotype showed moderate susceptibility, with an average disease severity of 45% under natural infection conditions in Nariño, Colombia. The variability in downy mildew incidence and severity levels observed across the 19 quinoa plots evaluated in this study could be related to differences in agronomic management practices and climatic conditions. Danielsen & Ames<sup>(19)</sup> reported that downy mildew can lead to significant defoliation and reduced crop yield when infections occur during early developmental stages, particularly under favorable environmental conditions. These conditions include cool temperatures ranging from 15 to 25 °C, relative humidity above 80%, and continuous precipitation.<sup>(35)</sup> Such conditions promote the germination of oospores and sporangia, therefore the multiplication and dissemination of the disease.<sup>(19)</sup>

The two quinoa samples collected in Bolívar and Totoró, showing downy mildew symptoms, exhibited morphometric characteristics of *Peronospora* sp., closely matching those described in studies from other quinoa-producing regions, although some slight variations were noted. Choi *et al.*<sup>(36)</sup> reported sporangiophores as substraight as slightly curved in shape, ranging from 320 to 600 µm in length, which is somewhat longer than those observed in Cauca samples, the trunk base width ranged from 12 to 17 µm, with a length between 180 and 390 µm. These measurements show sporangiophores slightly longer than ours. In contrast, Kara *et al.*<sup>(37)</sup> reported sporangiophores with lengths comparable to this study, ranging from 110 to 370 µm, and trunk base width of around 15 µm, which further supports our findings. The observed characteristics of the sporangia from Colombian isolates also corresponds with those reported for *P. variabilis*. Choi *et al.*<sup>(36)</sup> documented ellipsoidal sporangiophores with length from 24.5 and 35.0 µm, and widths from 20.5 to 27.3 µm. Similarly, Kara *et al.*<sup>(37)</sup> described ellipsoidal to obovoid or napiform shapes, with lengths from 25 to 35 µm and widths from 22.5 to 27.5 µm. Danielsen & Ames<sup>(19)</sup> reported oval sporangia ranging from 25.7 to 31.9 µm in length and 19.3 to 24.3 µm in

width.

The mature oospores showed morphological characteristics that partially correspond to those described by Khalifa & Thabet,<sup>(38)</sup> who reported dark brown structures with thick walls and an average diameter ranging from 30 to 55 µm in the pericarp of *C. quinoa* seeds. Similarly, Kara *et al.*<sup>(37)</sup> documented globose oospores in foliar tissue of *C. album*, with diameters between 20 and 30 µm, closely aligning with the dimensions recorded in this study. In contrast, greater morphological variability was noted when compared to the findings of El-Assiuty *et al.*<sup>(39)</sup> who described smaller globose or ovoid oospores (14–22 µm) in reproductive tissues such as the perianth, pericarp, seed coat, perisperm, and embryo cotyledons of *C. quinoa* seeds.

Molecular identification using concatenated analysis of ITS and COX2 fragments confirmed the identity of *P. variabilis*. Phylogenetic analysis revealed two distinct clades corresponding to lineages previously reported of *P. variabilis* based on COX2 sequences. This finding aligns with the geographical distribution of the pathogen, as noted in studies by Testen *et al.*,<sup>(40)</sup> Nolen *et al.*<sup>(41)</sup> and Fondevilla *et al.*<sup>(9)</sup> Colombian isolates appear closely related to those from Ecuador and Bolivia but distant from Spain and most USA isolates. To better understand the population dynamics of *P. variabilis*, more genes should be included in future. However, as an obligate pathogen, obtaining sequences from the same organism is challenging. Recent studies have shown that COXI is promising for intra-specific variations studies.<sup>(9)</sup> These results underscore the potential of using phylogenetic analysis based on concatenated sequences for molecular identification and populational studies of *P. variabilis*. Moreover, the analyses suggest that the species most closely related to *P. variabilis* is *P. chenopodii-fictifoli*, a relationship that has been previously highlighted during the recent rediscovery of this species.<sup>(42)</sup>

In pathogenicity tests, the inoculation of *P. variabilis* on detached leaves successfully reproduced the symptoms of downy mildew, thereby confirming this microorganism as the causal agent of the disease. This method has been widely utilized in various studies to assess the resistance of different quinoa genotypes to downy mildew, with symptoms typically appearing between 3 and 12 days post-inoculation (dpi), reflecting varying levels of susceptibility.<sup>(19,43)</sup>

In Colombia, quinoa cultivation operates primarily under traditional, non-technical agricultural practices, demonstrating significant potential for integration into family farming schemes and food security programs.



However, the limited information available regarding disease identification and agronomic and phytosanitary management of quinoa presents a considerable challenge to ensuring sustainable production.

The high incidence and severity of downy mildew recorded in the municipalities of Bolívar, La Vega, Silvia, and Totoró (Cauca) underscore the urgent need to enhance diagnostic and monitoring capabilities for this pathogen. It is essential to gain a deeper understanding of its biology, interaction with environmental factors, and propagation dynamics across various quinoa-producing regions. Additionally, establishing local research programs to develop management strategies tailored to specific agro-climatic conditions is crucial for improving the sustainability of quinoa cultivation.

## CONCLUSION

Morphometric and molecular analyses, together with pathogenicity tests, confirmed *P. variabilis* as the causal agent of downy mildew in quinoa crops in Colombia. The identification of symptoms associated with the disease, the confirmation of the pathogen, and the assessment of incidence and severity offer a strong foundation for implementing effective management strategies, thereby supporting sustainable and efficient quinoa production in the region.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest regarding the execution of this research or the publication of this manuscript.




## DATA AVAILABILITY

The nucleotide sequences generated and analyzed during this study have been deposited in the NCBI Nucleo-




tide database and are publicly available under the accession numbers PQ350322–PQ350335 for the ITS region and PQ351605–PQ351618 for the COX2 locus. These data can be accessed directly via the NCBI website: <https://www.ncbi.nlm.nih.gov/nucleotide/>




## AUTHOR CONTRIBUTIONS

**Conceptualization:** Diana Milena Rodríguez-Mora .

**Formal analysis:** Diana Milena Rodríguez-Mora , Kevin Alejandro Rodríguez-Arévalo , Luz Natalia Martínez-Caballero .





**Funding acquisition:** Diana Milena Rodríguez-Mora .



**Investigation:** Diana Milena Rodríguez-Mora , Isabel Cristina Ramirez-Paz , Luz Natalia Martínez-Caballero .

**Methodology:** Diana Milena Rodríguez-Mora , Isabel Cristina Ramirez-Paz , Luz Natalia Martínez-Caballero .

**Project administration:** Diana Milena Rodríguez-Mora .

**Supervision:** Diana Milena Rodríguez-Mora .

**Writing – original draft:** Diana Milena Rodríguez-Mora , Isabel Cristina Ramirez-Paz , Kevin Alejandro Rodríguez-Arévalo , Luz Natalia Martínez-Caballero .

**Writing – review & editing:** Diana Milena Rodríguez-Mora , Kevin Alejandro Rodríguez-Arévalo .

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**Supplementary Table 1.** *Peronospora* spp. sequences included in the phylogenetic analysis of Colombian isolates

Sample Name	Taxon	Host	Origin – Location	Genbank accession		Reference
				ITS	COX2	
BDM2A	<i>Peronospora variabilis</i>	<i>Chenopodium quinoa</i>	Bolivia - Cochabamba Department	KF269538	KF269649	(40)
BDM3D	<i>P. variabilis</i>	<i>C. quinoa</i>	Bolivia - Cochabamba Department	KF269541	KF269645	(40)
BDM5B	<i>P. variabilis</i>	<i>C. quinoa</i>	Bolivia - Cochabamba Department	KF269544	KF269652	(40)
BDM14A	<i>P. variabilis</i>	<i>C. quinoa</i>	Bolivia - Oruro Department	KF269578	KF269626	(40)
BDM16E	<i>P. variabilis</i>	<i>C. quinoa</i>	Bolivia - Oruro Department	KF269575	KF269666	(40)
BDM17B	<i>P. variabilis</i>	<i>C. quinoa</i>	Bolivia - Chuquisaca Department	KF269580	KF269667	(40)
ECPV4	<i>P. variabilis</i>	<i>C. quinoa</i>	Ecuador - Chimborazo Province	KF269605	KF269677	(40)
ECPV8	<i>P. variabilis</i>	<i>C. quinoa</i>	Ecuador - Chimborazo Province	KF269606	KF269678	(40)
ECDM10	<i>P. variabilis</i>	<i>C. quinoa</i>	Ecuador - Cotopaxi Province	KF269594	KF269636	(40)
ECDM19	<i>P. variabilis</i>	<i>C. album</i>	Ecuador - Tungurahua Province	KF269596	KF269638	(40)
ECDM53	<i>P. variabilis</i>	<i>C. quinoa</i>	Ecuador - Chimborazo Province	KF269599	KF269640	(40)
LV1	<i>P. variabilis</i>	<i>C. quinoa</i>	USA - Lancaster County, PA	KF269611	KF269683	(40)
LV2	<i>P. variabilis</i>	<i>C. quinoa</i>	USA - Lancaster County, PA	KF269612	KF269684	(40)
LV3	<i>P. variabilis</i>	<i>C. quinoa</i>	USA - Lancaster County, PA	KF269613	KF269685	(40)
RS	<i>P. variabilis</i>	<i>C. quinoa</i>	USA - Centre County, PA	KF269591	KF269673	(40)
S2018-1	<i>P. variabilis</i>	<i>C. album</i>	Spain - Córdoba	ON046672	ON092678	(9)
S2018-2	<i>P. variabilis</i>	<i>C. album</i>	Spain - Córdoba	ON046671	ON092677	(9)
S2018-4	<i>P. variabilis</i>	<i>C. quinoa</i>	Spain - Córdoba	ON046670	ON092675	(9)
S2019-1	<i>P. variabilis</i>	<i>C. album</i>	Spain - Córdoba	ON046669	ON092673	(9)
S2019-9	<i>P. variabilis</i>	<i>C. quinoa</i>	Spain - Seville	ON046661	ON092665	(9)
S2019-12	<i>P. variabilis</i>	<i>C. album</i>	Spain - Seville	ON046658	ON092662	(9)
S2019-14	<i>P. variabilis</i>	<i>C. quinoa</i>	Spain - Málaga	ON046656	ON092660	(9)
S2019-15	<i>P. variabilis</i>	<i>C. quinoa</i>	Spain - Villafranca, Córdoba	ON046655	ON092659	(9)
S2019-17	<i>P. variabilis</i>	<i>C. quinoa</i>	Spain - La Luisiana, Seville	ON046653	ON092657	(9)
S2019-20	<i>P. variabilis</i>	<i>C. quinoa</i>	Spain - Seville	ON046650	ON092654	(9)
S2019-24	<i>P. variabilis</i>	<i>C. album</i>	Spain - Córdoba	ON046646	ON092650	(9)
S2019-26	<i>P. variabilis</i>	<i>C. quinoa</i>	Spain - Puente Viejo, Córdoba	ON046644	ON092648	(9)
S2019-27	<i>P. variabilis</i>	<i>C. quinoa</i>	Spain - Almodóvar del Río, Córdoba	ON046643	ON092647	(9)
B23	<i>P. variabilis</i>	<i>C. quinoa</i>	Colombia - Bolivar, Cauca	PQ350322	PQ351605	This study
B24	<i>P. variabilis</i>	<i>C. quinoa</i>	Colombia - Bolivar, Cauca	PQ350323	PQ351606	This study
B84	<i>P. variabilis</i>	<i>C. quinoa</i>	Colombia - Bolivar, Cauca	PQ350330	PQ351613	This study
B85	<i>P. variabilis</i>	<i>C. quinoa</i>	Colombia - Bolivar, Cauca	PQ350331	PQ351614	This study
B33	<i>P. variabilis</i>	<i>C. quinoa</i>	Colombia - Bolivar, Cauca	PQ350326	PQ351609	This study
B34	<i>P. variabilis</i>	<i>C. quinoa</i>	Colombia - Bolivar, Cauca	PQ350327	PQ351610	This study
B59	<i>P. variabilis</i>	<i>C. quinoa</i>	Colombia - Bolivar, Cauca	PQ350328	PQ351611	This study
B60	<i>P. variabilis</i>	<i>C. quinoa</i>	Colombia - Bolivar, Cauca	PQ350329	PQ351612	This study
B26	<i>P. variabilis</i>	<i>C. quinoa</i>	Colombia - Bolivar, Cauca	PQ350324	PQ351607	This study
B27	<i>P. variabilis</i>	<i>C. quinoa</i>	Colombia - Bolivar, Cauca	PQ350325	PQ351608	This study
T11	<i>P. variabilis</i>	<i>C. quinoa</i>	Colombia - Totoró, Cauca	PQ350332	PQ351615	This study
T12	<i>P. variabilis</i>	<i>C. quinoa</i>	Colombia - Totoró, Cauca	PQ350333	PQ351616	This study
T117	<i>P. variabilis</i>	<i>C. quinoa</i>	Colombia - Totoró, Cauca	PQ350334	PQ351617	This study
T118	<i>P. variabilis</i>	<i>C. quinoa</i>	Colombia - Totoró, Cauca	PQ350335	PQ351618	This study
GLM51002	<i>P. boni-henrici</i>	<i>C. bonus-henricus</i>	Austria - Tirol	KP330867	KP330687	(25)
KUS-F20063	<i>P. cheonopodii-ambrosioides</i>	<i>C. ambrosioides</i>	Korea - Jeju	MT734673	MT731360	(42)
KUS-F18857	<i>P. cheonopodii-ficifolii</i>	<i>C. ficifolium</i>	Korea - Namyangju	AY211018	MT731363	(42)
BP118	<i>P. rumicis</i>	<i>Rumex acetosella</i>	Denmark - Ulvshale	KP330811	KP330631	(25)