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Isolation and molecular identification of contaminating fungi in vegetable seeds: Topito type sweet pepper (*Capsicum sinense*); winter squash (*Cucurbita moschata*) and eggplant (*Solanum melongena*) of economic importance in the Colombian Caribbean Coast

Aislamiento e identificación molecular de hongos contaminantes en semillas de hortalizas: Ají dulce tipo Topito (*Capsicum sinense*), ahuyama (*Cucurbita moschata*) y berenjena (*Solanum melongena*) de importancia económica en la Costa Caribe colombiana

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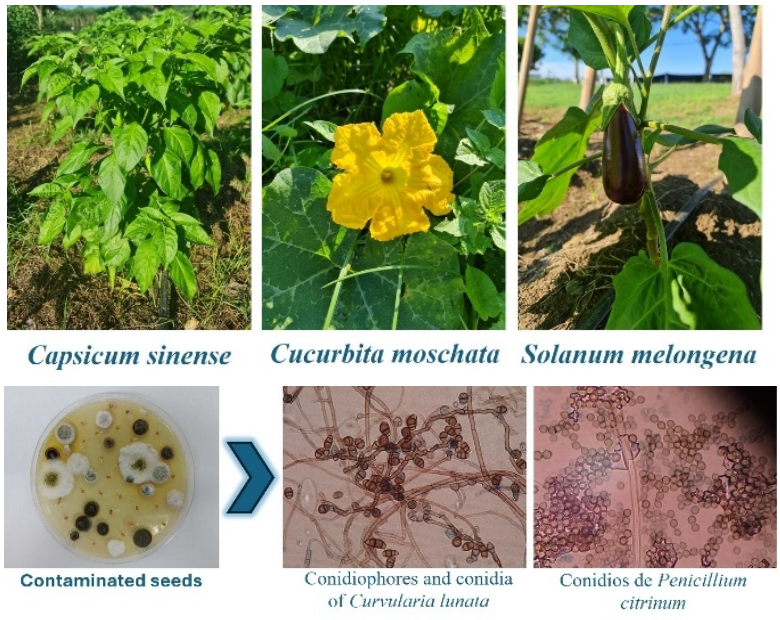

Field seed production and seed health test of Topito type sweet pepper (*Capsicum sinense* Jacques); winter squash (*Cucurbita moschata* Duch) and eggplant (*Solanum melongena* L.).
Photos: C. Espitia-Romero

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Short title: CONTAMINATING FUNGI IN VEGETABLE SEEDS: TOPITO TYPE SWEET PEPPER, WINTER SQUASH AND EGGPLANT

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ABSTRACT

Fungal contamination of Topito-type sweet pepper (*Capsicum chinense*), eggplant (*Solanum melongena*), and winter squash (*Cucurbita moschata*) seeds in the Colombian Caribbean represents a major threat to small-scale horticultural production. This study aimed to identify fungal contaminants through seeds incubation on potato dextrose agar, followed by morphological and molecular characterization via amplification of the ITS1 and ITS4 regions of the internal transcribed spacer (ITS). A total of 32 fungal isolates were obtained, classified into 25 morphotypes. Amplicon sizes ranged from 497 to 1,452 bp, with identity percentages between 89 and 100% compared to GenBank reference sequences. Winter squash PST1 exhibited contamination in 37% of seeds, predominantly by *Fusarium equiseti* (26%), while Topito-type sweet pepper DTP1 showed 37% contamination, with *Penicillium citrinum* (16%) as the most frequent species. Eggplant varieties Bv1 and Bv2 presented a higher diversity of fungal contaminants, including *Curvularia lunata*, *Fusarium oxysporum*, and *Schizophyllum commune*. The identified fungi included saprophytic and potentially pathogenic species associated with plant necrosis and seed deterioration. Morphological analysis revealed distinct macroscopic and microscopic traits, such as *Fusarium* spp.'s canoe-shaped macroconidia and *Penicillium citrinum*'s brush-like conidial structures. This study provides new insights into fungal contamination in vegetable seeds, identifying previously unreported species in these crops. The findings highlight the need to refine production protocols to enhance contaminant prevention. Additionally, the presence of fungi in stored seeds underscores the importance of proper humidity and temperature control during storage to prevent pathogen proliferation and ensure seed quality, contributing to more sustainable horticultural production in the region.

Additional key words: fungus; pathogenic fungus; seed; vegetable; seed quality; plant health.

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RESUMEN

La contaminación fúngica de semillas de ají dulce tipo Topito (*Capsicum chinense*), berenjena (*Solanum melongena*) y ahuyama (*Cucurbita moschata*) en el Caribe colombiano supone una amenaza para la producción hortícola a pequeña escala. Este estudio identificó contaminantes fúngicos incubando semillas en agar de dextrosa de papa y realizando una caracterización morfológica y molecular mediante la amplificación de las regiones ITS1 e ITS4. Se aislaron 32 cepas fúngicas, clasificadas en 25 morfotipos, con amplicones que oscilaron entre 497 y 1.452 pb y porcentajes de identidad del 89 al 100% en comparación con secuencias de GenBank. La ahuyama PST1 presentó contaminación en el 37% de las semillas, por *Fusarium equiseti* (26%), mientras que el ají dulce Topito DTP1 mostró el mismo porcentaje, siendo *Penicillium citrinum* (16%) la especie más prevalente. Las berenjenas Bv1 y Bv2 evidenciaron mayor diversidad fúngica, incluyendo *Curvularia lunata*, *Fusarium oxysporum* y *Schizophyllum commune*. Los hongos identificados comprenden especies saprófitas y patógenas, asociadas con la necrosis de plantas y el deterioro de semillas. El análisis morfológico reveló rasgos macroscópicos y microscópicos distintivos, como las macroconidias en forma de canoa de *Fusarium* spp. y las conidias en forma de brocha de *P. citrinum*. Este estudio aporta nuevos conocimientos sobre la contaminación fúngica en semillas de hortalizas, identificando especies no reportadas previamente. Además, resalta la necesidad de optimizar los protocolos de producción y almacenamiento para prevenir contaminantes, regulando la humedad y la temperatura para evitar la proliferación de patógenos.

Palabras clave adicionales: hongo; hongo patógeno; semilla; hortaliza; calidad de la semilla; sanidad vegetal.

INTRODUCTION

Vegetable production in the Colombian Caribbean has traditionally been a small-scale activity, carried out mainly in backyards and home gardens under family farming systems (Ordóñez, 2009). Despite the region's favorable climate, vegetable production has not been extensive (Martínez Reina *et al*., 2020), especially for crops such as sweet pepper (*Capsicum sinense* Jacq.), eggplant (*Solanum melongena* L.) and winter squash (*Cucurbita moschata* Duch.), which are fundamental to the local diet due to their nutritional contribution of vitamins, minerals and antioxidants (FAO and CIRAD, 2021).

Most of the seed used in these crops comes from non-conventional systems, such as seed exchange between producers at local and regional levels, reuse of seed from previous harvests, or on-farm seed production using selected local varieties or those acquired from other regions (Martínez Reina *et al*., 2020). These practices expose seeds to a higher risk of pathogen contamination, which can lead to reduced germination, reduced plant vigor and, ultimately, significant economic losses for smallholder farmers (Mohanto *et al*., 2019).

This practice, common in small-scale horticultural production, exposes crops to an increased risk of disease cycle after cycle due to the persistence and spread of fungi, increasing the risk of infection regionally (El-Baky and Amara, 2021). In addition to the diseases they cause, fungi release toxins that inhibit the growth and development of plants, which in turn further aggravate their condition and affect their production (Peng *et al*., 2021). This situation is exacerbated by the lack of knowledge in the identification of fungal diseases and the appropriate control measures to treat and prevent them in the future. Although several studies have focused on pathogens on leaves, stems and roots, but research on fungi on seeds is limited (Boixel *et al*., 2024), especially in the Colombian Caribbean context.

Therefore, identification of contaminating fungal species in vegetable seeds is essential to understand their pathogenic potential and to develop management strategies that minimize the risk of disease transmission (Iquebal *et al*., 2021). The process of identification through the study of morphology, i.e. by the observation and recognition of certain phenotypic characteristics, necessitates the expertise of mycologists who specialize in this field. The complexity of this process can be further compounded by the variations in fungal morphology that occur under diverse growth conditions (Raja *et al*., 2017; Salem-Bango *et al*., 2023). Consequently, over the past two decades, there has been a notable increase in the utilization of DNA sequence-based methodologies for species identification (Satam *et al*., 2023).

This is where the use of internal transcribed spacer sequencing (ITS), has proven effective in rapidly identifying fungi at the genus and species level, facilitating the implementation of more accurate and sustainable control measures. The ITS region is a DNA sequence that has been shown to be a reliable marker for the identification of fungi (Toledo *et al*., 2013; Raja *et al*., 2017; Bradshaw *et al*., 2023; Chinnasamy *et al*., 2023; Kauserud, 2023). This region is characterised by its ease of amplification, even from minimal amounts of genetic material (Kauserud, 2023), and its capacity to facilitate the comparison of the sequence of an unidentified

fungus with a substantial number of ITS nucleotide sequences stored in international databases such as ESBL or NCBI (Raja *et al*., 2017; Chinnasamy *et al*., 2023; Kauserud, 2023). This ITS region has facilitated the characterization of numerous fungi; however, it is subject to certain limitations, related to interspecific and intraspecific molecular variations, and technical errors during laboratory that can be confused with natural molecular variations (Bradshaw *et al*., 2023; Kauserud, 2023).

Despite the advent of high-throughput sequencing techniques capable of generating millions of short reads simultaneously, such as the Illumina Miseq platform, enabling the characterization of complex and diverse fungal communities, these methods continue to rely on ITS sequencing as a fundamental tool for taxonomic resolution, i.e., to ensure accurate fungal identification, the ITS1 or ITS2 regions had to be amplified and analyzed separately, due to their high variability between species and their conserved flanking regions, which facilitate primer design and amplification (Kauserud, 2023).

In the current genomic era, where advances in sequencing technologies continue to push boundaries, ITS sequencing remains essential for fungal identification, particularly in contexts where whole genome sequencing is not yet feasible due to cost or technical constraints Gudisa *et al*., 2024). This is particularly important in low- and middle-income countries, where ITS-based methods continue to provide cost-effective and reliable means of fungal identification and biodiversity assessment.

Promoting the use of high-quality seeds free of fungal contamination is essential not only to improve crop productivity in the Colombian Caribbean, but also to ensure food security and the well-being of rural communities. For this reason, ensuring seed health not only has an impact on agricultural success, but also contributes to protection of the ecological balance and conservation of the natural resources that support these production systems.

The identification of contaminating fungi in seeds is essential to improve agricultural productivity in regions such as the Colombian Caribbean. The aim of this study was to isolate and molecularly identify the fungi present in the seeds of Topito type sweet pepper (*C. sinense*), winter squash (*C. moschata*) and eggplant (*S. melongena*), crops of great economic importance in the region. This will allow us to increase our knowledge of the fungal diversity associated with these seeds, facilitating the development of more specific and efficient management strategies.

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MATERIALS AND METHODS

Study site

The present work was carried out between 2023 and 2024, in the Agricultural Microbiology, Molecular Genetics and Plant Production Laboratories of the Tibaitatá Research Center of the Colombian Agricultural Research Corporation (AGROSAVIA), Mosquera (Colombia), latitude N 4º41'43.1349", longitude W 74º12'18.7666", and altitude 2,600 m.

Plant material

The sanitary quality of the seed of three materials of the species winter squash PST1; Topito type sweet pepper DTP1; from the seed multiplication field located in Magdalena - Zona Bananera and two cultivars of eggplant Bv1 and Bv2 from the seed multiplication field located in Cerete - Cordoba were evaluated.

Incubation

Determination of fungal contaminants was carried out using the agar plate incubation methodology described by Mancini *et al*. (2016) and Marcinkowska (2002). Briefly, seeds were arranged equidistantly on 150 mm diameter Plastic Petri Dishes with sterilized potato dextrose agar (PDA) (Oxoid, Thermo Scientific Cat. No. CM0139B o 10197602) supplemented with chloramphenicol (Sigma Aldrich Cat. No. C0378) and 0.01% Triton X-100 (Millipore, Cat. No. 108603) to inhibit bacterial growth and limit fungal development, respectively.

After seed placement (n=50 for Topito type sweet pepper and eggplant n=23 for winter squash), the plates were incubated for 7 d in a climate camera (Panasonic Climate Camera MLR-352) for temperature and light control under conditions of 8 h of light and 16 h of darkness at a temperature of 30±8°C, except for the winter squash seeds, which were incubated at 25±3°C and a relative humidity of 90±5%.

Isolation and identification of fungal contaminants

At the end of the incubation period, isolates grown on seeds were characterized microscopically (Agrios, 2005) up to 40X in a microscope (Carl Zeiss Axiolab 5) by observing characteristic reproductive structures with taxonomic keys (Barnett and Hunter, 1972). Each of

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the recovered isolates was seeded on PDA plates (Oxoid, Thermo Scientific Cat. No. CM0139B o 10197602) and incubated at 25°C for 8 d. After the incubation period, the biomass produced was collected in a 2 mL microcentrifuge tube, macerated with a sterile pistil and frozen in liquid nitrogen. Subsequently, genomic DNA was extracted according to the method described by Griffith and Shaw (1998). The integrity of the extracted DNA was assessed by 0.8% agarose gel electrophoresis (PanReac AppliChem, Cat. No. A8963) stained with SYBR Safe gel stain (Invitrogen, Thermo Fisher Scientific, Cat. No. S33102), and its concentration and purity indices were determined by spectrophotometry using a NanoDrop 2000 (Thermo Scientific).

For molecular identification of the fungal isolates, the ITS1 and ITS4 regions of the internal transcribed spacer (ITS) were amplified by polymerase chain reaction (PCR) using the primers ITS1 Forward (5′-CTTGGTCATTTAGAGGAAGTAA-3′) (Gardes and Bruns, 1993) and ITS4 Reverse (5′-TCCTCCGCTTATTATTGATATGC-3′) (White *et al*., 1990). For molecular identification of the fungal isolates, the ITS1 and ITS4 regions of the internal transcribed spacer (ITS) were amplified by polymerase chain reaction (PCR), using the primers ITS1 Forward (5′-CTTGGTCATTTAGAGGAAGTAA-3′) (Gardes and Bruns, 1993) and ITS4 Reverse (5′-TCCTCCGCTTATTATTGATATATGC-3′) (White *et al*., 1990). Each amplification reaction included 5.0 µL DNA, 0.5 µL Taq polymerase (GoTaq Promega), 0.4 µL of each primer, 6.0 µL dNTP, 6.0 µL $MgCl_2$, 10 µL buffer and 21.7 µL sterile water, for a total volume of 50 µL. The thermal cycler (Bio-Rad T100) was programmed with the following PCR conditions: one activation cycle at 95°C for 2 min, followed by 35 cycles of denaturation at 94°C for 1 min, banding at 55°C for 30 s, and extension at 72°C for 1 min, with a final extension step at 72°C for 10 min. PCR products, with an approximate length of 550 bp, were visualized by electrophoresis on a 1 % agarose gel electrophoresis (PanReac AppliChem, Cat. No. A8963) stained with SYBR Safe gel stain (Invitrogen, Thermo Fisher Scientific, Cat. No. S33102). After verification of the amplified fragments, they were sequenced by the Sanger method.

The resulting sequences were compared with the corresponding ITS1-5.8S- ITS2 region of the sequences from the GenBank database of the National Center for Biotechnology Information (NCBI). The isolated fungal biotypes were preserved in PDA medium at -4°C (three copies of each isolate), and in cryovials in cryopreservation solution 20% glycerol (PanReac AppliChem Cat. No. 131339) and 0.5% peptone special (Oxoid, Thermo Scientific Cat. No. LP0072B) at -80°C.

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RESULTS AND DISCUSSION

The isolation and identification of fungal contaminants from the seeds of Topito type sweet pepper (*C. sinense*), winter squash (*C. moschata*), and eggplant (*S. melongena*) allowed the recovery of 32 pure fungal isolates. Based on their macroscopic and microscopic morphology, as well as the characters observed on the seeds, these isolates were grouped into 25 morphotypes. Of these, six morphotypes corresponded to winter squash PST1, seven to Topito type sweet pepper DTP1, eight morphotypes to eggplant to the variety Bv1 and four morphotypes to eggplant variety Bv2. Morphologically, the colonies exhibited diverse growth patterns, textures, and pigmentation. As observed in figure 2, which illustrates macroscopic colony morphology characteristics, and figure 3, which presents microscopic characteristics, *Rhizopus* sp. formed rapidly growing, dark gray, woolly colonies, with hyaline, coenocytic hyphae and sporangia connected by stolons with basal rhizoids (Ghosh *et al*., 2022). *Schizophyllum commune* displayed irregular, white, velvety colonies with hyaline, cenocytic masses of hyphae and sterile mycelium (Mahajan, 2022). *Penicillium citrinum* formed powdery, flat, round, and olive green at the top and white at the periphery colonies with septate, hyaline hyphae and conidial structures resemble brush-like arrangements (Nguyen *et al*., 2023). *Fusarium proliferatum* and *Fusarium oxysporum* exhibited pinkish, cottony colonies with cylindrical, septate microconidia and canoe-shaped macroconidia (Haapalainen *et al*., 2016). *Curvularia lunata* was distinguished by its radial, gray-to-brown colonies and pigmented, cylindrical, septate conidia with a swollen median cell (Priwiratama *et al*., 2024). *Fusarium equiseti* presented characteristic pinkish-orange colonies with abundant macroconidia (Hami *et al*., 2021), while *Aspergillus ochraceopetaliformis* exhibited a dense, powdery texture with yellowish conidia (Akter *et al*., 2023).

Megablast (Highly Similar Sequences) analysis of the sequences allowed the identification of the genus and species of the isolated fungi, with a percentage of identity that varied between 89% and 100% with the NCBI accessions (Tab. 1). The amplicon sizes obtained during sequencing ranged from 497 to 1,452 bp. The most notable differences in similarity scores were observed in *Fusarium equiseti* and *Fusarium oxysporum*, where *Fusarium equiseti* exhibited a closer match to known reference sequences, whereas *Fusarium oxysporum* showed a lower identity percentage (96.76%), suggesting possible genetic variability.

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Table 1. Results of the molecular identification of fungi isolated from the seeds of winter squash (*Cucurbita moschata*), Topito type sweet pepper (*Capsicum chinense*) DTP1, and eggplant (*Solanum melongena*) Bv1 and Bv2, based on ITS sequencing.

Specie	Morphotype	Identify fungi	Identity	Size (Pb)	Accession number similarity
Butternut squash (*Cucurbita moschata*) PST1	M01	*Fusarium equiseti* (Corda) Sacc.	99.78%	515	MF380715.1
	M02	*Fusarium equiseti* (Corda) Sacc.	89.01%	497	MN846297.1
	M03	*Penicillium herquei* Bainier & Sartory	99.80%	561	EU076963.1
	M04	*Fusarium graminearum* (Schwein.) Petch (1936)	100.00%	553	ON738580.1
	M05	*Trichoderma harzianum* Rifai (1969)	99.62%	590	MT065754.1
	M06	*Aspergillus ochraceopetaliformis* Batista et Maia	100.00%	615	MH857406.1
Topito type sweet pepper (*Capsicum chinense*) DTP1	M07	*Schizophyllum commune* Fries	99.82%	631	MN781968.1
	M08	*Penicillium citrinum* Thom, 1910	100.00%	546	MT597829.1
	M09	*Penicillium citrinum* Thom, 1910	99.57%	750	KY921944.1
	M10	*Penicillium citrinum* Thom, 1910	100.00%	523	MH282507.1
	M11	*Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg	99.56%	536	KY848358.1
	M12	*Fusarium oxysporum* Schltdl.	96.76%	552	MH665483.1
	M13	*Curvularia lunata* (Wakker) Boedijn	99.59%	541	MF380805.1
Eggplant (*S. melongena*) Bv1 and Bv2	M14	*Curvularia lunata* (Wakker) Boedijn	100.00%	534	MF380684.1
	M15	*Schizophyllum commune* Fries	99.83%	1,106	MN783217.1
	M16	*Penicillium ludwigii* Udagawa	100.00%	634	OR237696.1
	M17	*Talaromyces funiculosus* (syn. P. funiculosum Thom)	99.82%	1,452	MH859994.1
	M18	*Talaromyces amestolkiae*	100.00%	553	MW805716.

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	M19	*Cladosporium cladosporioides* (Fres.) de Vries	99.10%	531	MK518430.1
	M20	*Cladosporium cladosporioides* (Fres.) de Vries	100.00%	815	ON712443.1
	M21	*Penicillium camponotum* Visagie, David Clark & Seifert	99.80%	546	LC573502.1
	M22	*Aspergillus japonicus* Saito	100.00%	583	MT602615.1
	M23	*Cladosporium anthropophilum* Sandoval-Denis et al.	100.00%	502	MK111493.1
	M24	*Aspergillus aculeatus* Iizuka	100.00%	578	MT422091.1
	M25	*Fusarium equiseti* (Corda) Sacc.	99.56%	530	KU565733.1
	M26	*Rhizopus* sp. Ehrenb, 1820	99.80%	741	JN206366.1

Of the winter squash seeds analyzed, 63% were found to be free of contaminants, while the remaining 37% were infected by five fungal species, as shown in figure 1. These fungi were identified by their macroscopic morphology (Fig. 2), microscopic (Fig. 3) and molecular analysis. The species identified and their relative frequencies were *Aspergillus ochraceopetaliformis* (1%), *Fusarium equiseti* (26%), *Fusarium graminearum* (8%), *Penicillium herquei* (1%) and *Trichoderma harzianum* (1%).

In the case of Topito type sweet pepper DTP1, 63% of the seeds analysed showed no fungal contamination. However, eight fungal species were isolated from the remaining 37%, as shown in figure 1. The species identified and their frequency percentages were *Curvularia lunata* (2%), *Fusarium oxysporum* (3%), *Fusarium proliferatum* (6%), *Penicillium citrinum* (16%), *Penicillium* sp. (2%), *Rhizopus* sp. (3%), *Schizophyllum commune* (3%) and *Rhodotorula* sp. (2%).

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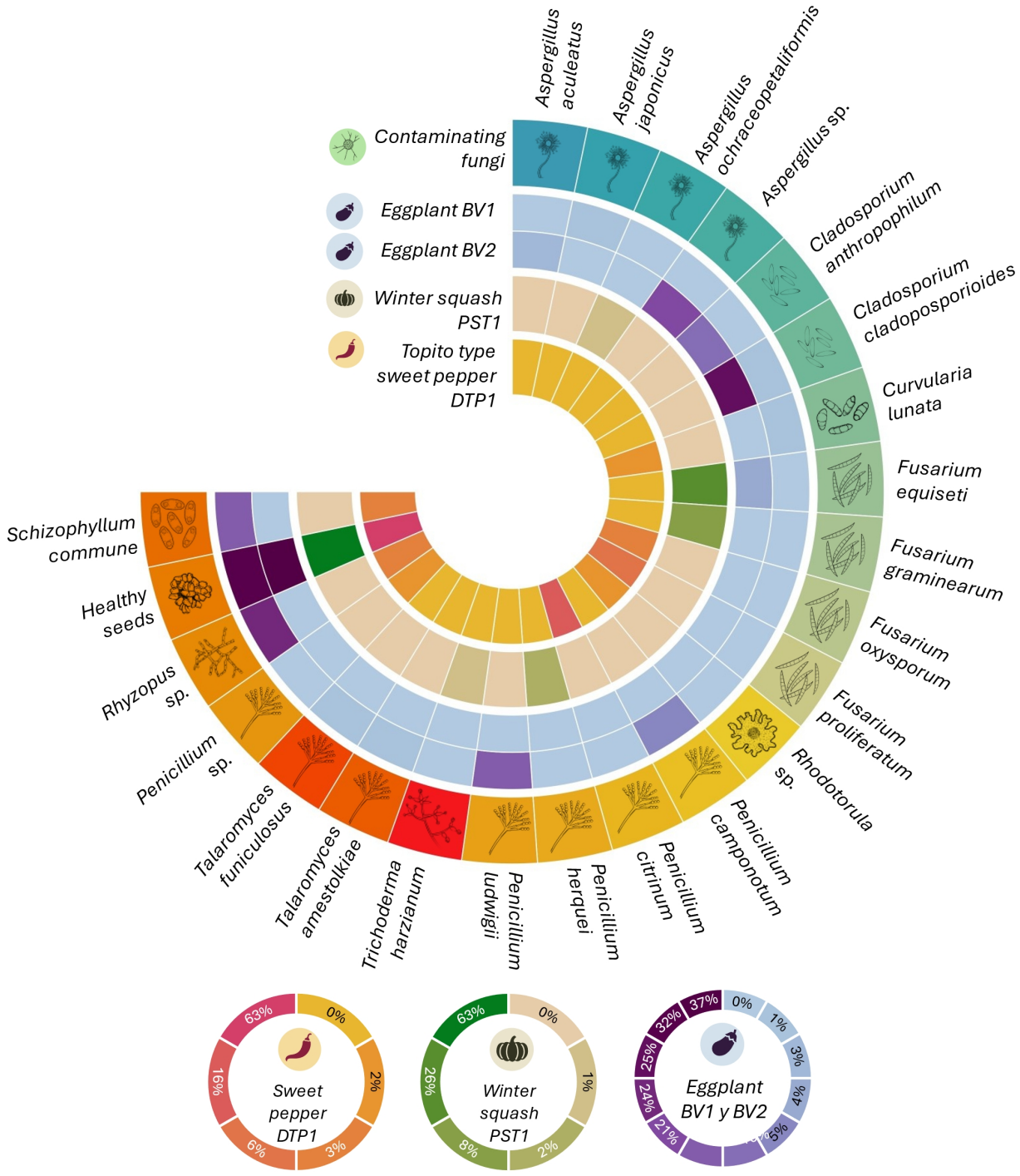

Figure 1. Circular heat map showing the distribution of contaminating fungi in vegetable seeds.

Analysis of eggplant seeds BV1 showed a fungal contamination rate of 68%, with nine species identified, of which *Rhizopus* sp. was the most abundant (24%) follow by *Schizophyllum*

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commune and *Penicillium ludwigii* (both with 17%), *Penicillium camponotum* (5%), and *Cladosporium cladosporioides and Cladosporium funicolosus* (both with 1%). In contrast, 63% of BV2 seeds showed contamination with a lower species diversity, dominated by *Cladosporium cladosporioides* (25%) and *Aspergillus* sp. (21%), *Cladosporium anthropophillum* (10%), *Fusarium equiseti* (4%) and *Aspergillus aculeatus* (3%). 32% of BV1 and 37% of BV2 showed no fungal presence (Fig. 1).

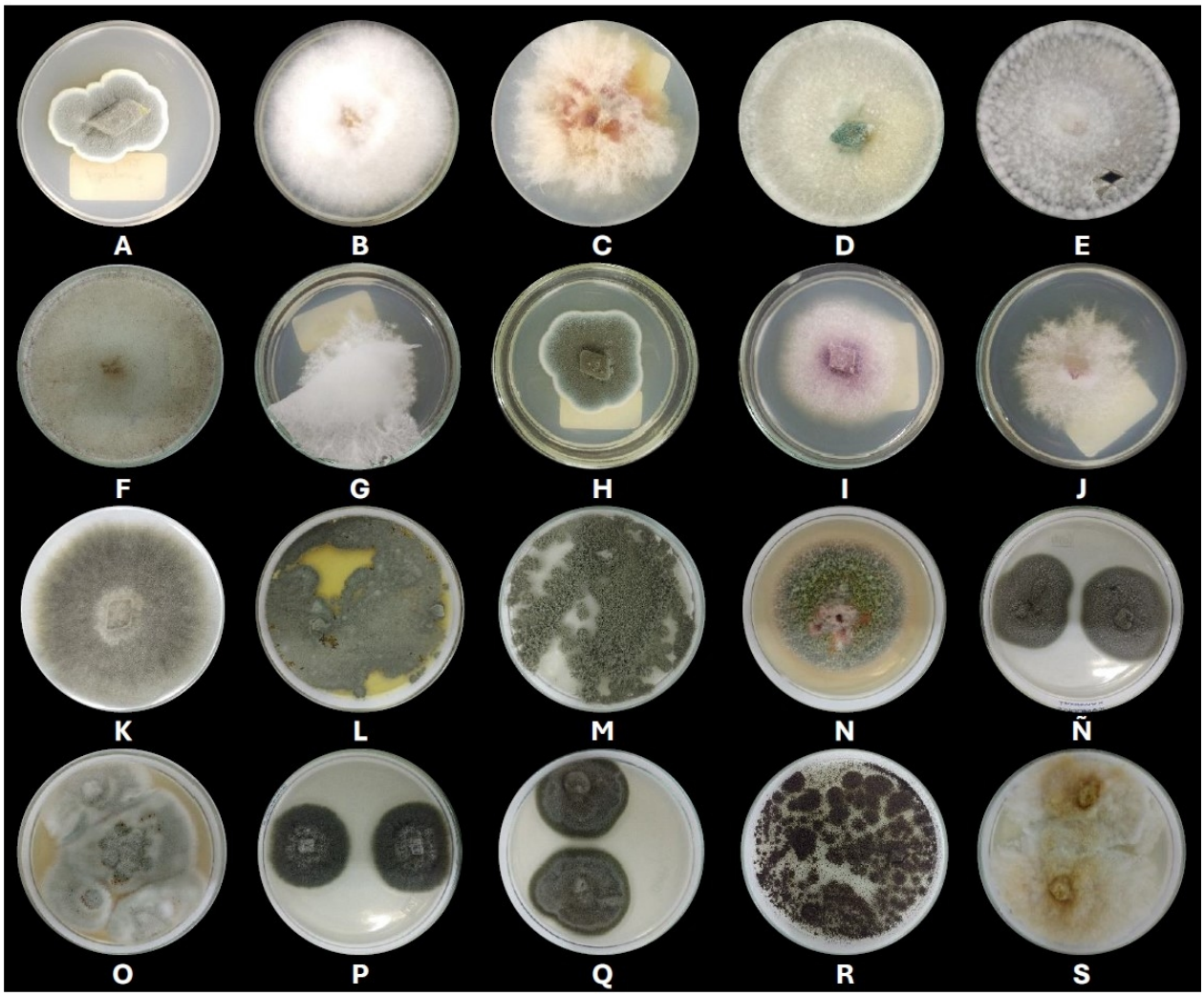

Figure 2. Fungi isolated from winter squash seeds: A) *Penicillium herquei*, B) *Fusarium equiseti*, C) *Fusarium graminearum*, D) *Trichoderma harzianum*, E) *Aspergillus ochraceopetaliformis.* Topito type sweet pepper DTP1 seeds: F) *Rhizopus* sp., G) *Schizophyllum commune*, H) *Penicillium citrinum*, I) *Fusarium proliferatum*, J) *Fusarium oxysporum*, K) *Curvularia lunata.* Eggplant Bv1: L) *Penicillium ludwigii*, M) *Talaromyces funiculosus*, N) *Talaromyces amestolkiae*, Ñ) *Cladosporium cladosporioides*, O) *Penicillium*

Patiño-Moscoso, M.A., J.A. Rivera-Avilez, K.V. Osorio-Guerrero, M.F. Valencia-Guerrero, and L.F. Sarmiento-Moreno. 2025. Isolation and molecular identification of contaminating fungi in vegetable seeds: Topito type sweet pepper (*Capsicum sinense*); winter squash (*Cucurbita moschata*) and eggplant (*Solanum melongena*) of economic importance in the Colombian Caribbean Coast. Revista Colombiana de Ciencias Hortícolas 19(1), e18634. Doi: https://doi.org/10.17584/rcch.2025v19i1.18634

***camponotum*. Eggplant Bv2: P) *Cladosporium anthropophilum*, Q) *Cladosporium cladosporioides*, R) *Aspergillus aculeatus*, S) *Fusarium equiseti*.**

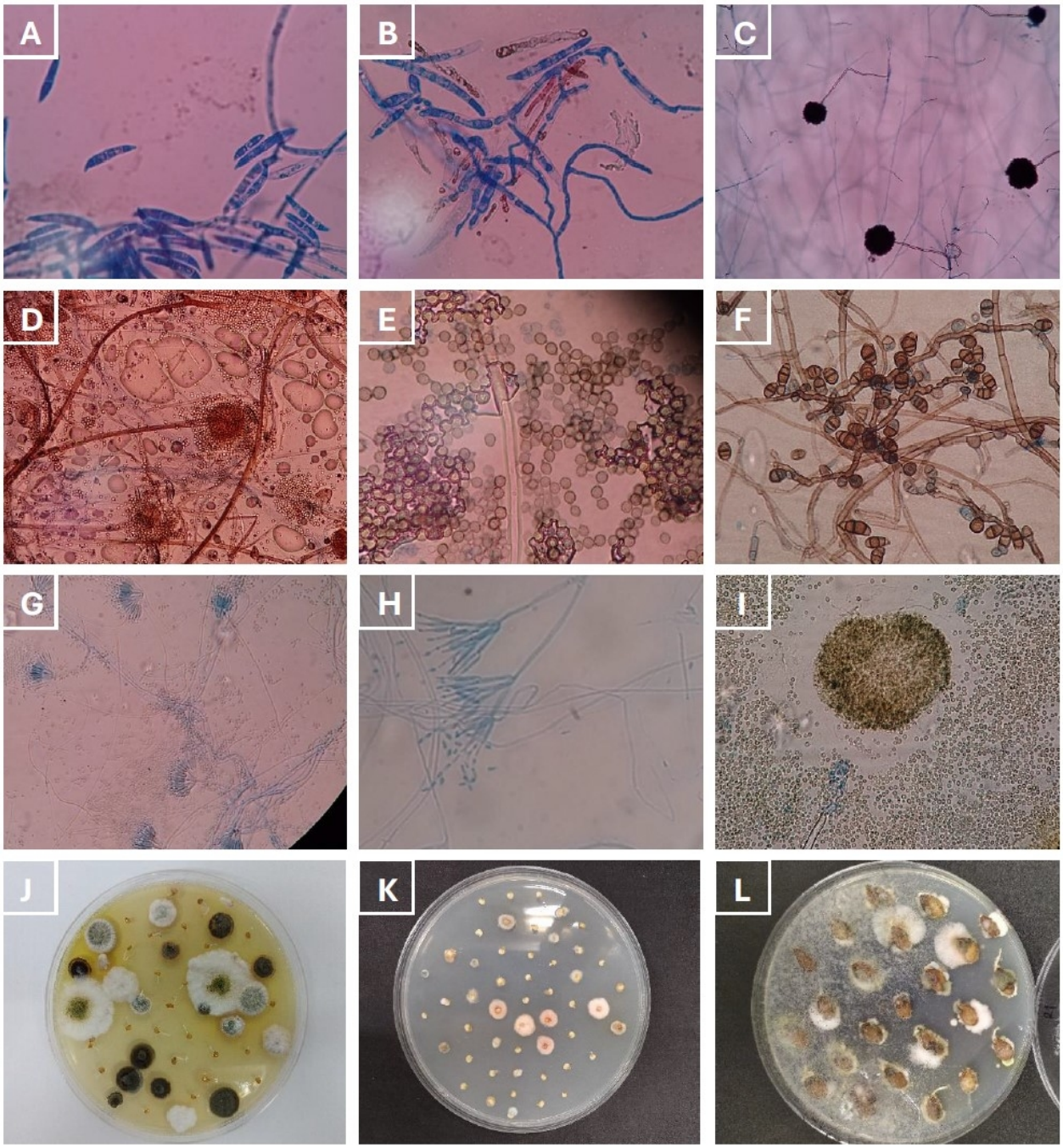

Figure 3. A) macroconidia of *Fusarium equiseti*, B) macroconidia of *Fusarium graminearum*, C) conidiophores of *Aspergillus ochraceopetaliformis*, D) esporangios de *Rhizopus* sp., E) conidios de *Penicillium citrinum*, F) conidiophores and conidia of *Curvularia lunata*, G) conidiophores and conidia of *Talaromyces funiculosis*, H) conidiphores and conidia *Talaromyces amestolkiae*, I) conidia of *Cladosporium*

***cladosporioides*, J) sanitary quality test on eggplant, K) sanitary quality test on Topito type sweet pepper DTP1, L) sanitary quality test on winter squash.**

The results of this study provide valuable information on the fungal contamination of vegetable seeds in the Caribbean region of Colombia. Accurate identification of these contaminants is essential for determining contamination sources, establishing critical control points in seed production, and ensuring seed quality. Detecting seed-borne pathogens is crucial for maintaining the health of seed stocks and preserving germplasm for future research and development (Vishunavat *et al*., 2023). A number of potentially pathogenic and saprophytic fungi were identified in the seeds of winter squash, Topito type sweet pepper DTP1 and eggplant. Potentially pathogenic and saprophytic fungi, including *Fusarium equiseti* and *Fusarium graminearum*, were identified in the seeds of winter squash. These species have a notable association with root, stem and fruit rot and necrosis in cucurbits (Demir *et al*., 2023). Fusarium *equiseti* has been reported to cause root rot and seedling wilt in courgettes. Metabolites such as equisitin can adversely affect germination and seedling growth (Thomas and Tennant, 2019). Similarly, *Fusarium graminearum* can cause growth inhibition and wilting in peppers (Engalycheva *et al*., 2024). It is likely that some of the fruits from which seeds were collected had dry rot in the areas in contact with the soil, as these fungi can enter the seeds through lesions in the fruit (Wang *et al*., 2019).

Fungi such as *Penicillium* sp., *Rhizopus* sp., *Fusarium oxysporum*, *Fusarium proliferatum* and *Curvularia* sp. were identified in Topito type sweet pepper DTP1. *Penicillium* sp. and *Rhizopus* sp. are considered environmental fungi that can contaminate seeds at any post-harvest stage. In order to control their presence, it is necessary to carry out air quality tests in the processing and storage areas, checking surfaces, air ducts and containers (Liu *et al*., 2021a).

Fusarium oxysporum and *Fusarium proliferatum* are pathogens that have been reported on Topito type sweet pepper DTP1, causing wilting and soil problems (Abada and Ahmed, 2014). *Curvularia lunata*, on the other hand, has been associated with leaf spot on habanero peppers (Cruz-Cerino et al., 2023). The presence of these fungi suggests the need to assess the sanitary conditions during seed production, and to confirm whether the fruit or seeds have been in contact with the soil, which could facilitate contamination.

Fungi such as *Rhizopus* sp., *Schizophyllum commune*, *Penicillium ludwigii*, *Talaromyces amestolkiae* and *Talaromyces funiculosus* were found on eggplant seeds. In particular, *Rhizopus* sp., known to cause storage rot, is particularly prevalent on cultivar BV1 (Liu *et al*., 2024a). This fungus can be introduced during the harvesting and transport process, where bruising of the fruit facilitates entry of the pathogen. In addition, *Rhizopus* spores can be airborne and survive in the soil for months (Silva *et al*., 2020).

Penicillium and *Aspergillus* genus also affect the quality of stored seeds and can negatively affect germination and mycotoxin production (Lezcano *et al*., 2015; Maldonado and Romero, 2022). Although *Aspergillus aculeatus* has not been reported as a seed pathogen, it can promote plant growth and mitigate the negative effects of abiotic stress (Li *et al*., 2021). On the other hand, *Penicillium ludwigii* can mineralise nitrogen but has not been associated with pathogenicity on eggplant (Liu *et al*., 2024b).

Schizophyllum commune, although not previously reported on eggplant, is an important plant pathogen (Singh *et al*., 2020). Its spread is by basidiospores and is associated with damage to woody tissues, suggesting possible transmission through contaminated environments (Gálvez *et al*., 2021).

The fungi *Talaromyces amestolkiae* and *Talaromyces funiculosus*, although not reported as pathogens of eggplant, are associated with pathologies in maize. *T. amestolkiae* produces ochratoxin A, a mycotoxin of concern for human and animal health (Castillo, 2019). *T. funiculosus* has been reported to cause ear rot in maize (Liu *et al*., 2021b). This suggests possible cross-contamination from maize fields to eggplant seeds.

The fact that *Fusarium equiseti*, *Schizophyllum commune*, *Talaromyces amestolkiae* and *Talaromyces funiculosus* are associated with pathologies in maize indicates possible contamination of eggplant multiplication fields. Eggplant seed multiplication in Cordoba, Colombia, a region with high maize production, suggests that contamination may originate from these fields. Fungal spores can survive in crop residues and soil, contaminating subsequent crops (The CIMMYT Maize Program, 2004). In addition, weeds can act as hosts for these fungi, as observed in studies in Turkey (Manikandan *et al*., 2024).

The genus *Cladosporium* has been reported on eggplant, causing rotting of ripe fruits and leaf spots (Shafique *et al*., 2019). *Cladosporium cladosporioides* and *Cladosporium anthropophilum*

are common saprophytes that can become opportunistic pathogens under favorable conditions (Pérez and Espinosa, 2019; Tibpromma *et al*., 2019).

Finally, the presence of *Curvularia lunata* on eggplant can cause leaf spot and significantly affect crop quality (Atiq *et al*., 2022; Matrood, 2018). This species, along with other fungal pathogens, contributes to economic losses in eggplant production.

This research highlights the knowledge gap in fungal seed contamination in vegetables, a topic that has been poorly addressed (Zuparova *et al*., 2023). Fungal contamination in uncertified seeds is a major challenge as it can affect germination, growth and plant quality, with economic consequences for farmers (Baglan *et al*., 2020). The information generated shows the importance of assessing sanitary conditions and seed handling to mitigate the associated risks.

However, it is essential to bear in mind that research must consider the variability in the presence and virulence of pathogens under different environmental conditions. Interpretation of the pathogenicity of certain fungi should be made with caution, as their impact may vary according to the specific conditions of the crop and the environment.

CONCLUSION

This study has provided new knowledge on fungal contamination in vegetable seeds in the Caribbean region of Colombia, identifying fungal species that had not been previously reported in these crops. The results show that, although in some cases seed control and conservation practices have been adequate, in other species it is necessary to adjust production protocols to improve the prevention of contaminants. In addition, the prevalence of fungi in stored seeds highlights the importance of controlling humidity and temperature conditions during storage to avoid the proliferation and transfer of pathogens.

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Author's contributions: Patiño-Moscoso, M.A. designed the experiments, participated in the setting up of laboratory tests and data collection, performed the statistical analysis, interpreted the results, contributed to the writing, and took part in the final review of the manuscript. Rivera-Avilez, J.A. designed the experiments, participated in the setting up of laboratory tests and data collection, performed the statistical analysis, interpreted the results, contributed to the writing, and took part in the final review of the manuscript. Valencia-Guerrero, M.F. participated in the setting up of laboratory tests and data collection, interpreted the results, contributed to the writing, and took part in the final review of the manuscript. Osorio-Guerrero, K.V. participated in the setting up of laboratory tests and data collection, interpreted the results, contributed to the writing, and took part in the final review of the manuscript. Sarmiento-Moreno, L.F. interpreted the results, contributed to the writing, and took part in the final review of the manuscript.

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