REVISTA TEMAS AGRARIOS

ORIGINAL ARTICLE

Identification of sources of resistance to *Fusarium oxysporum* in *Physalis* sp. genotypes.

Identificación de fuentes de resistencia a *Fusarium oxysporum* en genotipos de *Physalis* sp.

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ABSTRACT

The resistance of Cape gooseberry to *Fusarium oxysporum* was evaluated in 70 accessions of *Physalis peruviana* and related taxa (*P. philadelphica, P. ixocarpa, P. floridana, P. pubescens, P. angulata, P. pruinosa, P. viscosa, P. mexicana, Nicandra physalodes,* and *Solanum auriculatum*). The accessions were obtained from different national and international collections, including accessions from the Colombian productive sector; these represented wild materials, commercial cultivars, native and foreign weeds, and commercial ecotypes from the main producing countries: Colombia, South Africa, Kenya, and Peru. The evaluation of resistance to *F. oxysporum* was carried out under greenhouse conditions using the most aggressive strain supplied by the *Fusarium* collection maintained by the molecular microbiology laboratory of the Center for Biotechnology and Bioindustry (CBB), currently in charge of the Working Collection of Microorganisms of Agrosavia, which was isolated from infected fields. The symptoms were monitored using a severity scale, containing 10 degrees and five categories. Data information obtained from daily evaluations was analyzed through a severity evaluation and different statistical analyses. The results identified one accession belonging to *Physalis peruviana* and two related taxa (*Physalis floridana* and *Solanum auriculatum*) as resistant to this pathogen. These accessions could be directly used in breeding programs, either as improved cultivars or as race-specific resistance donors for other Physalis peruviana genotypes.

Key words: Fungi disease, induced resistance, phytogenetic, plant breeding and vascular wilt.

RESUMEN

Se evaluó la resistencia de 70 accesiones de uchuva (*Physalis peruviana*) y taxones relacionados (*P. philadelphica*, *P. ixocarpa*, *P. floridana*, *P. pubescens*, *P. angulata*, *P. pruinosa*, *P. viscosa*, *P. mexicana*, *Nicandra physalodes* y *Solanum auriculatum*), a *Fusarium oxysporum* (Map 5). Las accesiones se obtuvieron de diferentes colecciones nacionales e internacionales, incluyendo del sector productivo colombiano. En las accesiones evaluadas se incluyeron materiales silvestres, cultivares comerciales, malezas nativas y foráneas y ecotipos comerciales de Colombia, Sudáfrica, Kenia y Perú. La evaluación de la resistencia se realizó en condiciones de invernadero utilizando la cepa más agresiva suministrada por la colección de *Fusarium* mantenida por el laboratorio de microbiología molecular del Centro de Biotecnología y Bioindustria (CBB), actualmente a cargo de la Colección de Trabajo de Microorganismos de Agrosavia, la cual fue aislada de campos infectados. Los síntomas fueron monitoreados usando una escala de severidad, que contenía 10 grados y cinco categorías. La información de los datos obtenidos de las evaluaciones diarias se analizó a través de una evaluación de severidad y diferentes análisis estadísticos. Los resultados identificaron una accesión perteneciente a *Physalis peruviana* y dos taxones relacionados (*Physalis floridana* y *Solanum auriculatum*) como resistentes a este patógeno. Estas accesiones podrían usarse directamente en programas de mejoramiento, ya sea como cultivares mejorados o como donantes de resistencia específicos de la raza para otros genotipos de *Physalis peruviana*.

Palabras clave: Enfermedades fúngicas, Resistencia inducida, fitogenético, fitomejoramiento y marchitez vascular.

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INTRODUCTION

Cape gooseberry (*Physalis peruviana*) is an exotic fruit belonging to the Solanaceae family, which is native to the tropical Andean region (Morillo-Coronado *et al*., 2018) and has currently spread to other parts of the world, such as Africa and India (Puentes *et al*., 2011). This tropical fruit has high contents of vitamin A, C and B-complex (Hassan *et al*., 2017) and minerals, and it is also known for its antioxidant and anticancer properties (Castro *et al*., 2020; Ballesteros-Vivas *et al*., 2019; Mariod, 2019). Colombia is the main Cape gooseberry producer worldwide (Ruiz *et al*., 2018). However, cape gooseberry has not increased its importance due to phytosanitary problems (Agronet, 2020). Some of the main problems are the fungal diseases (Moreno-Velandia, 2019; Chaves-Gómez *et al*., 2020ab), which generate huge crop losses, and consequently, a significant reduction in yield and quality products (Pulido, 2010).

In Colombia, within the fungal diseases this crop suffers, the most important is Fusarium wilt caused by *Fusarium oxysporum* (Cruz-Castiblanco *et al*., 2022). Yield losses caused by this pathogen reach 100% in the department of Cundinamarca, and 40 to 50% in the department of Boyacá, caused mainly by the transfer of infected material (Nuñez *et al*., 2014; García *et al*., 2021). This disease has also been reported in emergent centers of production in Colombia, such as the departments of Nariño and Antioquia (Osorio-Guarín *et al*., 2016; Ruiz *et al*., 2018). *Fusarium oxysporum* induces a wide variety of symptoms, including wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting, and damping-off. Within these symptoms, the most important is vascular wilt (Yánez *et al*., 2019; Roy *et al*., 2020). This problem is managed employing a wide array of approaches, such as fumigation (Edel-Hermann *et al*., 2019; ICA, 2020; Neto *et al*., 2020), soil sterilization using metham-sodium and/or methyl bromide (Reuveni *et al*. 1997), crop rotation (Sánchez, 2019), soil solarization (Nuñez *et al*., 2014; Arango *et al*., 2019), use of compost enriched with selected microorganisms (Carvalho *et al*., 2014) and biological control (Abdulridha *et al*., 2018). However, these methods are not 100% effective in the field for a long time (Jabnoun-Khiareddine *et al*., 2019), being imperative to find alternative control methods for this pathogen. The use of resistant varieties seems to be the most effective and desirable method (Maryani *et al*., 2019; Moreno-Miranda *et al*., 2020), due to its practical success in experiences reported in tomato (Walter cultivar) (Carvalho *et al*., 2014; Wu *et al*., 2019), lettuce (Garibaldi *et al*., 2004), and cucumber (Abro *et al*., 2019), among others. The use of resistant cultivars represents the sole effective and economically gainful measure for the control of Fusarium wilt in the field (Chacón *et al*., 2016).

Although collections of cape gooseberry germplasm with differential response to the disease have been studied in the country, reaching the identification of one and 16 SNPs, respectively, of genes with annotations in the defense / resistance response to pathogens (Enciso-Rodríguez *et al*., 2013; Osorio-Guarín *et al*., 2016; Orozco-Balbuena *et al*., 2021; Silva *et al*., 2022), and in Colombia there are two varieties of cape gooseberry recently released by Agrosavia (Núñez Zarantes *et al*., 2016ab; Núñez, 2020), no cultivar declares to be resistant or tolerant to *F. oxysporum* (Núñez Zarantes *et al*. 2016ab). In this sense, resistant cape gooseberry varieties to *F. oxysporum* are not known still. Given the magnitude of the problem and to be able to control the disease, the aim of this study is to find sources of resistance to this fungus from a comprehensive germplasm collection (Bonilla *et al*., 2009; Delgado-Bastidas *et al*., 2019), originating from the main producing centers of Colombia and the world, and using one of the most aggressive strains of *F. oxysporum* isolated from infected cape gooseberry fields (Pulido, 2010; Enciso-Rodríguez *et al*., 2013; Osorio-

Guarín *et al*., 2016). A standardized symptom evaluation scale specific to the *Fusarium oxysporum-Physalis peruviana* pathosystem was used, which allowed selecting resistant accessions and related taxa that will be used as promissory material for plant breeding programs.

MATERIALS AND METHODS

Plant Material

The resistance to *Fusarium oxysporum* was evaluated in 70 accessions of Physalis peruviana and related taxa (*P. philadelphica, P. ixocarpa, P. floridana, P. pubescens, P. angulatas, P. pruinosa, P. viscosa, P. mexicana, Nicandra physalodes*, and *Solanum auriculatum*) represented by wild materials, commercial cultivars, native and foreign weeds, and by commercial ecotypes coming from the main producing countries: Colombia, South Africa, Kenya, and Peru. The accessions were selected from different national and international collections, but mainly from the work collection of Corporación Colombiana de Investigación Agropecuaria - Agrosavia, according to their origin, ecotype, economic representativeness, and quality (Table 1).

Table 1. Selected species evaluated for resistance to *Fusarium oxysporum* with origin of germplasm collection.

Uchuva collection code Corpoica	Accession	Source	*Scientific name*	Code other institutions and initial passport	Country
09U005	06Uch0007	Universidad Nacional	*P. peruviana*		Colombia
09U012	06Uch0014	Universidad Nacional	*P. peruviana*		Colombia
09U021	06Uch0026	Universidad Nacional	*P. peruviana*		Colombia
09U022	06Uch0029	Universidad Nacional	*P. peruviana*		Colombia
09U031	06Uch0040	Universidad Nacional	*P. peruviana*		Colombia
09U045	06Uch0059	Universidad Nacional	*P. peruviana*		Colombia
09U047	06Uch0062	Universidad Nacional	*P. peruviana*		Colombia
09U056	ILS 3774	CI La Selva	*P. philadelphica*	350 - Guatemala	Guatemala
09U063	ILS 3781	CI La Selva	*P. philadelphica*	690 - Guatemala	Guatemala
09U071	ILS 3789	CI La Selva	*P. philadelphica*	1190 - Guatemala	Guatemala
09U078	ILS 3796	CI La Selva	*P. philadelphica*	1219 - Guatemala	Guatemala
09U086	ILS 3804	CI La Selva	*P. peruviana*	Uchuva ecuador carretera ambato	Ecuador
09U089	ILS 3807	CI La Selva	*P. peruviana*	Uchuva turbo San Pedro de Uraba	Colombia
09U093	ILS 3811	CI La Selva	*P. peruviana*	Uchuva la Ceja Antioquia	Colombia
09U099	ILS 3817	CI La Selva	*P. peruviana*	Uchuva reina, Mauricio Narvaez, Manizales, Caldas	Colombia

Uchuva collection code Corpoica	Accession	Source	*Scientific name*	Code other institutions and initial passport	Country
09U108	ILS 3826	CI La Selva	*P. peruviana*	Uchuva, Llanadas Abajo Sonson, Antioquia	Colombia
09U110	ILS 3828	CI La Selva	*P. peruviana*	Uchuva Plaza de Mercado Manizales caldas	Colombia
09U116	ILS 3834	CI La Selva	*P. peruviana*	Uchuva, denominado "Francia", Antioquia	Colombia
09U118	ILS 3836	CI La Selva	*P. peruviana*	Uchuva, Madrid, Cundinamarca	Colombia
09U124	ILS 3865	CI La Selva	*Nicandra physalodes*		Colombia
09U128	ILS 251	CI La Selva	*P. peruviana*		Colombia
09U132	ILS 502	CI La Selva	*P. peruviana*		Colombia
09U134	ILS 1432	CI La Selva	*P. peruviana*	874750001	Nepal
09U135	ILS 1433	CI La Selva	*P.angulata*	974750015	
09U136	ILS 1434	CI La Selva	*P. peruviana*	974750014	
09U138	ILS 1436	CI La Selva	*P. peruviana*	944750022	
09U139	ILS 1437	CI La Selva	*P.floridana Rydb.*	9047750219, donador original codigo S. 1168	
09U140	ILS 1438	CI La Selva	*P. peruviana*	944750164	
09U141	ILS 1439	CI La Selva	*P. floridana Rydb.*	944750023	
09U143	ILS 1441	CI La Selva	*P. pruinosa L.*	894750256	
09U144	ILS 1442	CI La Selva	*viscosa (anterior-P. fusco-maculata Dunal.)*	904750141, donador original codigo S.1370	
09U148	ILS 1446	CI La Selva	*P. philadelphica. Lam. ñ cv Golden Nugget*	924750193	
09U149	ILS 1447	CI La Selva	*P. philadelphica Lam*	944750024	
09U150	ILS 1448	CI La Selva	*P. pruinosa L.*	894750021	
09U151	ILS 1449	CI La Selva	*P. mexicana Molina exColla.*	904750216, Donador código original S. 0853	
09U153	ILS 1451	CI La Selva	*P. ixocarpa Brot. Ex Hornem*	PT0 COI 1219 (894740257)	
09U157	ILS 862	CI La Selva			Colombia
09U166	ILS 2026	CI La Selva	*P. peruviana*	Boy 36	Colombia
09U167	ILS 2075	CI La Selva	*P. peruviana*	Boy 13	Colombia
09U171	ILS 2173	CI La Selva	*P. peruviana*		Colombia
09U173	Physalis angulata	Universidad de Nariño	*P. angulata*		Colombia
09U176	Pi - 02	Universidad de Nariño	*Physalis ixocarpa*		Colombia
09U178	Solanum auriculatum	Universidad de Nariño	*Solanum auriculatum*		Ecuador
09U199	UN -31	Universidad de Nariño	*P. peruviana*		Colombia

Uchuva collection code Corpoica	Accession	Source	*Scientific name*	Code other institutions and initial passport	Country
09U201	UN - 22	Universidad de Nariño	*P. peruviana*		Colombia
09U202	UN - 5	Universidad de Nariño	*P. peruviana*		Colombia
09U210	Kenya - 1 (Libre Polinizacion)	Universidad de Nariño	*P. peruviana*		Kenya
09U213	Mondomo - Cauca	Universidad de Nariño	*P. peruviana*		Colombia
09U215	Ecotipo Kenia	Universidad de Nariño	*P. peruviana*		Colombia
09U216	Ecotipo Colombia	Universidad de Nariño	*P. peruviana*		Colombia
09U217	Ecotipo Perú	Universidad de Nariño	*P. peruviana*		Colombia
09U218	LA PILA-1-GRANADA/ CUND	CI Tibaitata	*P. peruviana*		Colombia
09U227	EL DIAMANTE-2-PASCA/CUND	CI Tibaitata	*P. peruviana*		Colombia
09U237	LA ESPERANZA-3-Cómbita/BOYACÁ	CI Tibaitata	*P. peruviana*		Colombia
09U249	EL ENCANTO-6-ARCABUCO/BOYACÁ	CI Tibaitata	*P. peruviana*		Colombia
09U254	DR.VICTOR-2	CI Tibaitata	*P. peruviana*		Colombia
09U261	LA EMERALDA-L1-T1-P3-GRANADA/CUND	CI Tibaitata	*P. peruviana*		Colombia
09U274	NOVACAMPO	CI Tibaitata	*P. peruviana*		Colombia
09U275	ILS 3767	CI La Selva	*P. peruviana*	C.I. FRUTIERREZ	Colombia
09U276	ILS 3957	CI La Selva	*P. peruviana*	UN-03	Colombia
09U277	ILS 3958	CI La Selva	*P. peruviana*	UN-24	Colombia
09U278	ILS 3959	CI La Selva	*P. peruviana*	UN-35	Colombia
09U279	ILS 3961	CI La Selva	*P. peruviana*	UN-43	Colombia
09U280	ILS 3962	CI La Selva	*P. peruviana*	UN-45	Colombia
09U281	ILS 3963	CI La Selva	*P. peruviana*	UN-49	Colombia
09U282	ILS 3964	CI La Selva	*P. peruviana*	UN-52	Colombia
09U283	ILS 3975	CI La Selva	*P. peruviana*	UNPU-120	Colombia
09U288	PI232077	National Plant Germplasm System(USDA/ ARS)	*P. peruviana*		South Africa
09U289	PI285705	National Plant Germplasm System(USDA/ ARS)	*P. peruviana*		Polonia
09U290	PI291561	National Plant Germplasm System(USDA/ ARS)	*P. peruviana*		India

* All accessions were named with a code corresponding to the reception year of the material, followed by the letter u (uchuva: Cape gooseberry in Spanish), and a consecutive number (e.g., 09u001).

Growth Conditions

Before sowing, all seeds were washed with a sodium hypochlorite solution in a proportion of 1% v/v for 1 min. A total of 36 seeds per accession were planted in pots filled with Canadian peat and vermiculite in a proportion of 50/50% (w/w) and placed in a greenhouse under controlled conditions (18 to 25 °C of temperature and 70 to 80% of relative humidity). From the selected accessions, seven were propagated *in vitro*, and four of these were used in this study: 09u005 (wild type), 09u215, 09u216 and 09u217 (ecotypes from Kenia, Colombia, and Peru, respectively). These accessions were evaluated with the pathogenic Map5 strain of *F. oxysporum*. Once the seedlings developed a pair of true leaves, they were transplanted into plastic pots with 255 g of substrate (soil-rice husk in a 3:1 ratio), kept under greenhouse conditions for four weeks before inoculation.

Fungal Strain

The pathogenic strain of *Fusarium oxysporum* (Map5) was supplied by the *Fusarium collection* maintained by the molecular microbiology laboratory of Centro de Biotecnología y Bioindustria (CBB), currently in charge of Agrosavia's Microorganism Work Collection, and with code 129 in the Alexander von Humboldt National Registry of Collections. This strain was isolated from infected Cape gooseberry fields in the department of Cundinamarca, Colombia, and showed a high incidence and severity under field and greenhouse conditions (Rodríguez, 2010).

Inoculum Production

The monosporic strain Map5, preserved in filter paper at -20 °C, was reactivated in PDA (Potato Dextrose Agar) medium for fifteen days at 28 °C. A piece of 1 cm^2 of agar with mycelium growth was cut and cultured in liquid PDB (Potato Dextrose Broth) for ten days at 28 °C under constant shaking (140 rpm). Subsequently, the inoculum was prepared, according to Namiki *et al.* (1994). The culture was filtered through sterile gauze and centrifuged in 50 mL Falcon® tubes for 10 min at 2000 x g. The conidial pellet was resuspended in sterile water and adjusted to the desired final concentration.

Inoculum Calibration

The appropriate concentration of inoculum was tested on a susceptible genotype using three different concentrations (10^3, 10^4, and 10^5 CFU mL^{-1}) (Martyn and McLaughlin, 1983; Caperton *et al.,* 1986). The plant inoculation experiments contained one replicate with 10 plants per concentration and three plants inoculated with sterile water as a control; these were repeated at least twice. All the experiments were carried out in a greenhouse under controlled conditions. Disease symptoms were monitored and scored daily for six weeks using a symptom evaluation scale proposed in this study (Table 2).

Table 2. Severity scale of symptoms for *Physalis peruviana* - *Fusarium oxysyporum* pathosystem.

Category Name	Degree	Severity (%)	Principal Symptoms	Phenotype
0. Resistance	0	= 0	Any visible symptoms	
	1	≤ 5	Few leaf damages, low to moderate discoloration	

Category Name	Degree	Severity (%)	Principal Symptoms	Phenotype
1. Low susceptible	2	≥ 6 - ≤10	Few lesions on the leaves; discoloration to light green with many dark greenareas	
	3	≥11-≤ 20	Few lesions, wilting on the edge of the leaf; shortly infected leaves, pale green or slightly yellow, with loss of turgor.	
2. Moderately susceptible	4	≥21-≤ 40	Evident infection in the leaves, discoloration to pale yellow, dried leaf edges, brown "burned", with little or no turgor, moderate infection of the stem; chlorosis.	
	5	≥41-≤ 60	Severe infection of leaves, yellow ocher shades, dried leaf to the midrib, "burned" edges; prostration of pedicel; total loss of turgor, moderate infection on the stem and purple color on the basis of the stem, chlorosis and / or necrosis.	
3. Susceptible	6	≥61-≤ 70	Severe lesions on the leaves, "Burn" overall, wilting, chlorosis, necrosis and / or defoliation premature; prostration of the stem, purple stain on the basis of the stem.	
	7	≥71-≤ 80	Dead leaves or 100% wilting, chlorosis, necrosis and / or premature defoliation, severe prostration, purple or violet stain on the stem.	
4. Highly susceptible	8	≥81 -≤ 90	Dead leaves, wilting, chlorosis, necrosis and severe defoliation, stem without force.	
	9	≥91 -≤ 100	Dead plant	

Inoculation and Symptoms Evaluation

Plant inoculation experiments contained two replicates with nine plants per isolate and nine plants inoculated with sterile water as a control; these were repeated at least twice to calibrate the inoculum. Eleven plants per accession were inoculated with the conidial suspension, and one plant was dipped in sterile water to evaluate the resistance of the germplasm with the resulting optimal concentration. The inoculation was carried out using the root-dip method, according to Namiki *et al*. (1994). The roots were dipped in 500 mL of spore suspension for 3 min and then transplanted into the same pots. The pathogen was isolated from vascular bundles of some inoculated plants and was confirmed morphologically as *F. oxysporum*, according to Lesllie and Summerell (2006). External symptoms were scored two weeks after inoculation for 45 days. The severity degree of the disease was registered daily using a scale of symptoms (Table 2). This scale was built based on other scales suggested by CIAT (1987), Enciso-Rodríguez *et al.* (2013), Yadav *et al.* (2017) and Pabón-Villalobos *et al*. (2022).

Data Analysis

Resulting data from the inoculum calibration test were analyzed by logistic regression, considering the last day of evaluation, i.e., 45 days after the first symptom (dafs), using the SAS software package (Cody, 2018). For this analysis, a binary scale was established considering nine symptom degrees, giving a value of 0 (healthy) to plants showing symptom degrees from 0 to 3, and giving a value of 1 to plants showing symptom degrees from 4 to 9. From this data, the probability of occurrence of dead plants within each concentration was calculated. Data were also assessed through an analysis of variance (ANOVA) using a completely randomized design with concentration as the only factor; means were compared by Tukey's multiple comparison test (P=0.05) using the SAS software package, version 9.1.3. Data information obtained from daily evaluations of 70 accessions of *Physalis peruviana* and related taxa were analyzed first through a severity analysis, then carrying out conglomerate and clustering analyses, and finally, employing a chi-square test. The severity analysis for each accession was done from the average value of the data obtained at 45 dafs (Reuveni *et al*., 1997). For the conglomerate and clustering analyses, disease incidence (DI) and disease severity were taken as informative variables. DI was estimated as the percentage of dead plants (i.e., degree 9 - highly susceptible in the severity scale) over the total number of inoculated plants per accession. Disease severity was estimated as the scale degree most frequently obtained per accession. For the conglomerate analysis, a principal component analysis was carried out using the PROC PRINCOMP procedure of the SAS 9.1.3 package. For the clustering analysis, the Ward algorithm was used, selecting six groups and employing the PROC CLUSTER procedure of SAS 9.1.3. (Cody, 2018). The chi-square test was done using the binary scale employed in the inoculum calibration analysis. Frequencies of 0 > 80% were considered as resistant. The results obtained from the severity and clustering analyses, as well as from the chi-square test, were compared to designate resistant accessions.

RESULTS AND DISCUSSION

Symptom Severity Scale

A diagrammatic symptom severity scale corresponding to each category was disposed from 1,800 photographic records obtained from the evolution of the symptoms during 45 dafs

Inoculum Calibration

At thirty-three dafs, the inoculum concentration of 104 CFU mL^{-1} showed a probability of 100%

dead plants. Also, during the 45 days of the evaluation period, this concentration showed to be the most aggressive, suggesting a difference in the effectiveness of the infection (Figure 1).

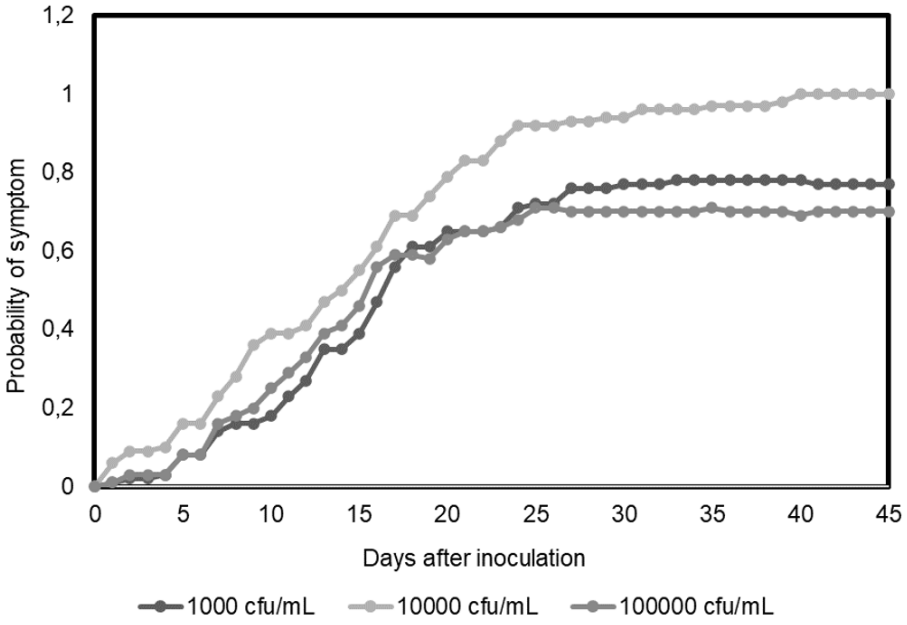

Figure 1. Logistic regression used for inoculums calibration showed that 104 CFU mL^{-1} was the most effective concentration at early times after inoculation.

The design was significant with an F-value of 8.13 and a p-value of 0.0004 (Mean square: model= 1.9305; error= 0.2374). Tukey's test showed differences in treatments, which brings together in one group the concentrations of 103 to 105 CFU mL^{-1}, with an average lower than the average concentration of 104 CFU mL^{-1}.

Selection of Resistant Germplasm

According to the severity analysis, three accessions were resistant (09u139, 09u178, and 09u279), and 67 were susceptible (95.71%). From the latter, 39 were highly susceptible (55.71%), 15 were susceptible (21.43%), eight were moderately susceptible (11.43%), and five were low susceptible accessions (7.14%) (Table 3).

The clustering analysis showed six clusters using a semi-partial R-square (SPRSQ) of 0.0004 and an R-square (RSQ) of 0.999. From this analysis, nine accessions (12.86%) were estimated as resistant (09u047, 09u063, 09u071, 09u078, 09u139, 09u173, 09u176, 09u178, and 09u279), and 61 accessions (87.14%) as susceptible. Within the susceptible accessions, four were estimated as low susceptible (5.71%), seven as moderately susceptible (10.0%), 12 as susceptible (17.14%), and 36 as highly susceptible (51.43%). In this analysis, we included two accessions as tolerant, which did not correspond to any degree of the severity scale (Figure 2). The chi-square analysis identified four accessions as resistant: 09u063, 09u071, 09u139 and 09u178 (5.71%), with a percentage of occurrence of 75% by event 0 (resistant). At 70% of occurrence by event 0, accession 09u279 was included. This analysis was significant with a p-value of <0.0001.

Table 3. The severity analysis of resistance of the accession of cape gooseberry.

Accession	Average	Evaluation
09u005	6	Susceptible
09u012	9	Highly susceptible
09u005	6	Susceptible
09u012	9	Highly susceptible
09u021	9	Highly susceptible
09u022	9	Highly susceptible
09u031	8,91	Highly susceptible
09u045	7,82	Susceptible
09u047	3,91	Low susceptible
09u056	8,91	Highly susceptible
09u063	2	Low susceptible
09u071	2	Low susceptible
09u078	4,82	Moderately susceptible
09u086	7,36	Susceptible
09u089	9	Highly susceptible
09u093	8,18	Highly susceptible
09u099	9	Highly susceptible
09u108	8,18	Highly susceptible
09u110	9	Highly susceptible
09u116	8,18	Highly susceptible
09u118	9	Highly susceptible
09u124	5,54	Moderately susceptible
09u128	7,45	Susceptible
09u132	9	Highly susceptible
09u134	5	Moderately susceptible
09u135	7,09	Susceptible
09u136	8,18	Highly susceptible
09u138	4,36	Moderately susceptible
09u139	0,64	Resistant
09u140	8,18	Highly susceptible
09u141	9	Highly susceptible
09u143	9	Highly susceptible
09u144	9	Highly susceptible
09u148	8	Highly susceptible
09u149	9	Highly susceptible
09u150	9	Highly susceptible
09u151	9	Highly susceptible
09u153	9	Highly susceptible
09u157	9	Highly susceptible
09u166	9	Highly susceptible
09u167	8,27	Highly susceptible
09u171	9	Highly susceptible
09u173	3,82	Low susceptible
09u176	2,18	Low susceptible
09u178	0,54	Resistant
09u199	9	Highly susceptible
09U201	8,83	Highly susceptible
09u202	9	Highly susceptible
09u210	7,82	Susceptible
09u213	8,09	Highly susceptible
09u215	7,83	Susceptible
09u216	4,08	Moderately susceptible
09u217	4,5	Moderately susceptible
09u218	6,42	Susceptible
09u227	6,45	Susceptible
09u237	9	Highly susceptible
09u249	6,36	Susceptible
09u254	5,27	Moderately susceptible
09u261	5,24	Moderately susceptible
09u274	6,91	Susceptible
09u275	6,2	Susceptible
09u276	9	Highly susceptible
09u277	7,5	Susceptible
09u278	9	Highly susceptible
09u279	1,33	Resistant
09u280	9	Highly susceptible
09u281	6,42	Susceptible
09u282	8,9	Highly susceptible
09u283	8	Highly susceptible
09u288	6,45	Susceptible
09u289	8,45	Highly susceptible
09U290	8,09	Highly susceptible

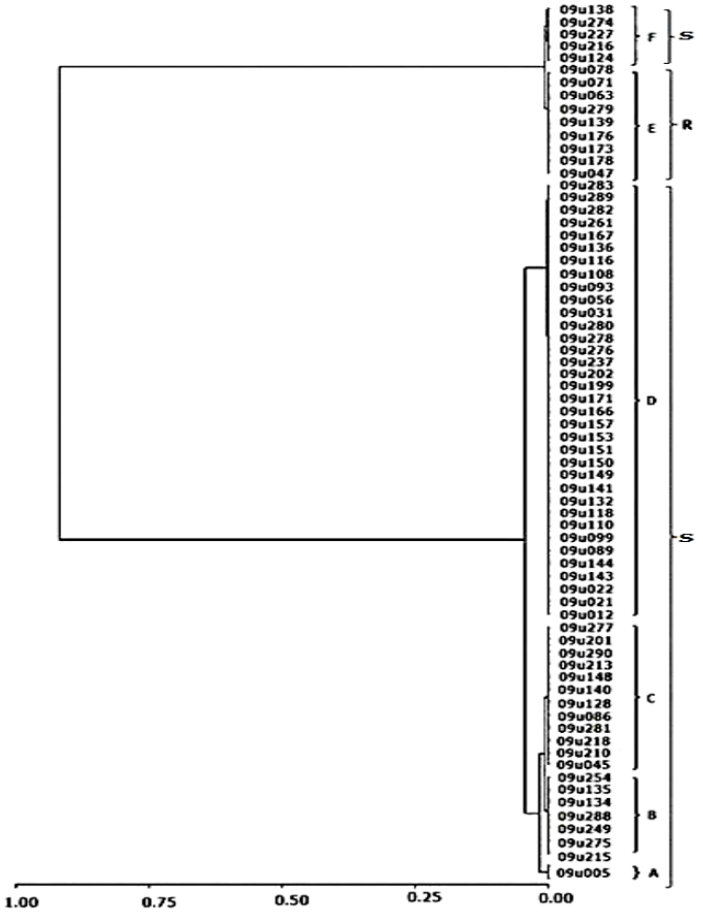

Figure 2. Dendrogram deduced from incidence and severity analysis. Two differentiated groups (S, Susceptible and R, Resistant) were conformed according to the category of the symptom: A, Tolerant; B, Moderately Susceptible; C, Susceptible; D, Highly Susceptible; E, Resistant; F, Low Susceptible.

The four statistical analyses showed consistency in the results. Consistently, three accessions were resistant (09u279, 09u178, and 09u139) and five accessions were considered as low susceptible, at least in two different analyses (09u216, 09u176, 09u173, 09u071, and 09u063) (Table 4).

Fusarium wilt on Cape gooseberry is the most destructive disease and the main limiting factor in the continuous worldwide expansion of cropped areas with Cape gooseberry (Cháves-Gómez *et al*., 2020c). Currently, conventional methods of control, including chemical control with fungicides, are ineffective and harmful to the environment. Asan alternative to this problem, the identification and use of resistant germplasm is the desirable strategy for a better control of the disease (Mayorga-Cubillos *et al*., 2019; Lagos-Brubano *et al*., 2021; Odhiambo *et al*., 2021). Given the magnitude of the problem, and in order to control the disease, we found promising materials held in various germplasm collections of *Physalis peruviana*., including related taxa, after artificial inoculation with one of the most aggressive strains of *F. oxysporum*, which was isolated from the most affected Cape gooseberry cropping area in Colombia. The germplasm collection used in this study comprised materials with a very

diverse geographical origin, ranging from wild native plants to materials from North American and European collections; all of these were considered as being unexplored and promising sources of resistance. Given the extensive collection available for searching sources of resistance to *Fusarium oxysporum*, it was necessary to create a highly efficient inoculation technique and a symptom evaluation scale.

Table 4. Results obtained from data analysis using the four methodologies to assess resistance or susceptibility of gooseberry germ plasm to Fusarium oxysporum. These results only include resistance and low susceptibility categories. Chi square test is based on a 70% occurrence of the event 0.

Category	Scale of severity	Cluster analysis	Dendrogram	Chi-square
			09u227	
		09u216	09u216	
	09u176	09u176		
	09u173	09u173		
			09u138	
			09u124	
Low susceptible		09u089		
		09u078		
	09u071	09u071		
	09u063	09u063		
	09u047			
		09u005		
	09u279	09u279	09u279	09u279
	09u178	09u178	09u178	09u178
			09u176	
			09u173	
Resistant	09u139	09u139	09u139	09u139
			09u078	
			09u071	09u071
			09u063	09u063
			09u047	

According to the inoculation data, the methodology using the dip-root immersion technique allowed evidencing the symptoms produced by *F. oxysporum*, suggesting that this method ensured the penetration of the pathogen without any type of injury (puncture or injection) and consequently, the activation of the first line of defense. The results obtained using dip-root immersion and an inoculum pressure of 104 CFU mL^{-1}, indicated that the inoculation system with the Map5 isolate of *F. oxysporum* under greenhouse conditions was reliable for the identification of resistant individuals among the selected germplasm. This concentration has also been reported in studies of pathosystems, such as *Fusarium oxysporum* f. sp. lycopersici-*Solanum lycopersicum* (Tello and Lacasa, 1988), and *F. oxysporum* f. sp. *basilicum*-Ocimum basilicum (Reuveni *et al.,* 1997). Although 104 CFU mL^{-1} is a low concentration in contrast to other reports (Mandal *et al*. 2008), this concentration is closer to values of propagules found in natural infection conditions (Agrios, 2008). The current study allowed standardizing a symptom evaluation scale specific to the *Fusarium oxysporum-Physalis peruviana*

pathosystem. Ten degrees and five categories could be established by visual scoring according to the severity percentage. This scale allowed differentiating consistently, resistant and susceptible accessions to *F. oxysporum*.

The best estimation of the resistance level was reached 45 days after inoculation. This scale allowed selecting resistant accessions and related taxa that could be used like promissory material for plant breeding programs. The number of categories in this scale was adequate for a rapid and accurate evaluation of the symptoms, solving the problem of estimation, occurring in impractical scales with a low number of classes, with low resolution or with an excessive number of classes (Gayosso *et al*., 2021; Pabón-Villalobos *et al*., 2022). The visual material used as support in the scale was very important to eliminate the arbitrariness of the selected intervals between classes, which usually are used to categorize different degrees of severity. This assures an accurate and reproducible evaluation system during the practical implementation of the symptom evaluation scale (Yadav *et al*., 2017).

In this study, five categories (resistant, low susceptible, moderately susceptible, susceptible and highly susceptible) were assigned according to the severity of the disease and were used to establish resistance and susceptibility to *F. oxysporum* under greenhouse conditions. In this sense, such categories have been used to study other pathosystems involving *Fusarium* with satisfactory results (Caperton *et al*., 1986; Martyn and McLaughlin, 1983; Leitao *et al*., 2020). Although some authors are more flexible in assigning categories of resistance or susceptibility to this pathogen (Cardona y Castaño, 2019; Giraldo-Betancourt *et al*., 2020; Fischer *et al*., 2021), in this study we considered within each category, narrow ranges of disease severity to avoid false identification of resistant accessions.

CONCLUSIONS

The analysis of the symptom evaluation data showed that only three could be classified as resistant, one corresponding to *Physalis peruviana* (09u279) and two corresponding to related taxa, i.e., *Physalis floridana* (09u139) and *Solanum auriculatum* (09u178), The 95,7% of the tested genotypes were susceptible (low, moderately and highly), however, accession 09u047, considered in this study as having low susceptibility or being resistant, according to the analysis, would be considered as a possible source of resistance to vascular wilt.

The resistance level observed in the related taxa showed a phenotype that ranged from 0% damage (total resistance) in *Solanum auriculatum* (09u178) to 5% damage in *Physalis floridana* (09u139). In contrast, the resistant phenotype observed in the *P. peruviana* accession was not absolute as it showed slight chlorosis (less than 5% of the leaf area) and loss of turgor; nevertheless, neither of the symptoms progressed during the 45 evaluation days, not even five months later. During this time, the plants always had a very good size with any symptom of the disease.

The results obtained suggest that the accessions resistance to the tested strain, although these are not commercial, can be a potential source for used in breeding programs through the development of hybrids (Lagos-Burbano *et al*., 2020; Lagos-Burbano *et al*., 2021), however, it is necessary to evaluate these accessions with other common *F. oxysporum* strains coming from different geographical origins.

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Author contributions

Victor Camilo Pulido-Blanco, I did revision of the source primary and secundary of information on fusarium oxysporium on Cape gooseberry, development the methodology, collect of data experimental and support the writing; and Carlos Felipe Gonzalez-Chavarro, I did writing- original draft preparation, I did the search for information to build the discussion, later, I did review and editing for submit to the journal.

Competing Interest

We, Victor Camilo Pulido-Blanco and Carlos Felipe Gonzalez-Chavarro, declare that the text has not been nor will be sent for publication to another journal during the evaluation of the manuscript, acceptance process, and, if applicable, its publication. I manifest that there are no economic or other relationships that could lead to a conflict of interest in this study. Furthermore, I am responsible for attending the indications, corrections, and suggestions of the evaluators.

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