# A Cytogenomic Analysis Reveals a New *Fusarium fujikuroi* Species Associated with Lemongrass (*Cymbopogon citratus*)

Tomás Cavaco[1,2,*, †], Catarina Gomes-Domingues [3,2, †], Filipe Gonçalves [2]

[1] Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Quinta do Marquês, 2780-159 Oeiras, Portugal
[2] Instituto Superior de Agronomia (ISA), Universidade de Lisboa, 1349-107 Lisboa, Portugal; fpgonc@gmail.com
[3] Linking Landscape, Environment, Agriculture and Food (LEAF), Research Centre, Associated Laboratory TERRA, Instituto Superior de Agronomia (ISA), Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal; catarinaagomes01@gmail.com
* Correspondence: tomasfcavaco@gmail.com
† These authors contributed equally to the work.

## Introduction

The *Fusarium* genus (Ascomycota: Hypocreales) is composed of ubiquitous filamentous fungi, comprising 20 species complexes. These ascomycetes are among the most economically relevant fungal plant pathogens, therefore being a threat to crop health and farmers' financial sustainability. Furthermore, *Fusarium* spp. have secondary biosynthetic pathways capable of producing harmful mycotoxins, which lessens food safety worldwide. The *Fusarium fujikuroi* species complex (FFSC) is divided into three distinct clades (American, African, and Asian), encompassing more than 50 different species. Several causal agents of plant diseases belong to FFSC, such as *Fusarium circinatum*, *F. fujikuroi*, *F. sacchari*, and *F. verticillioides*.

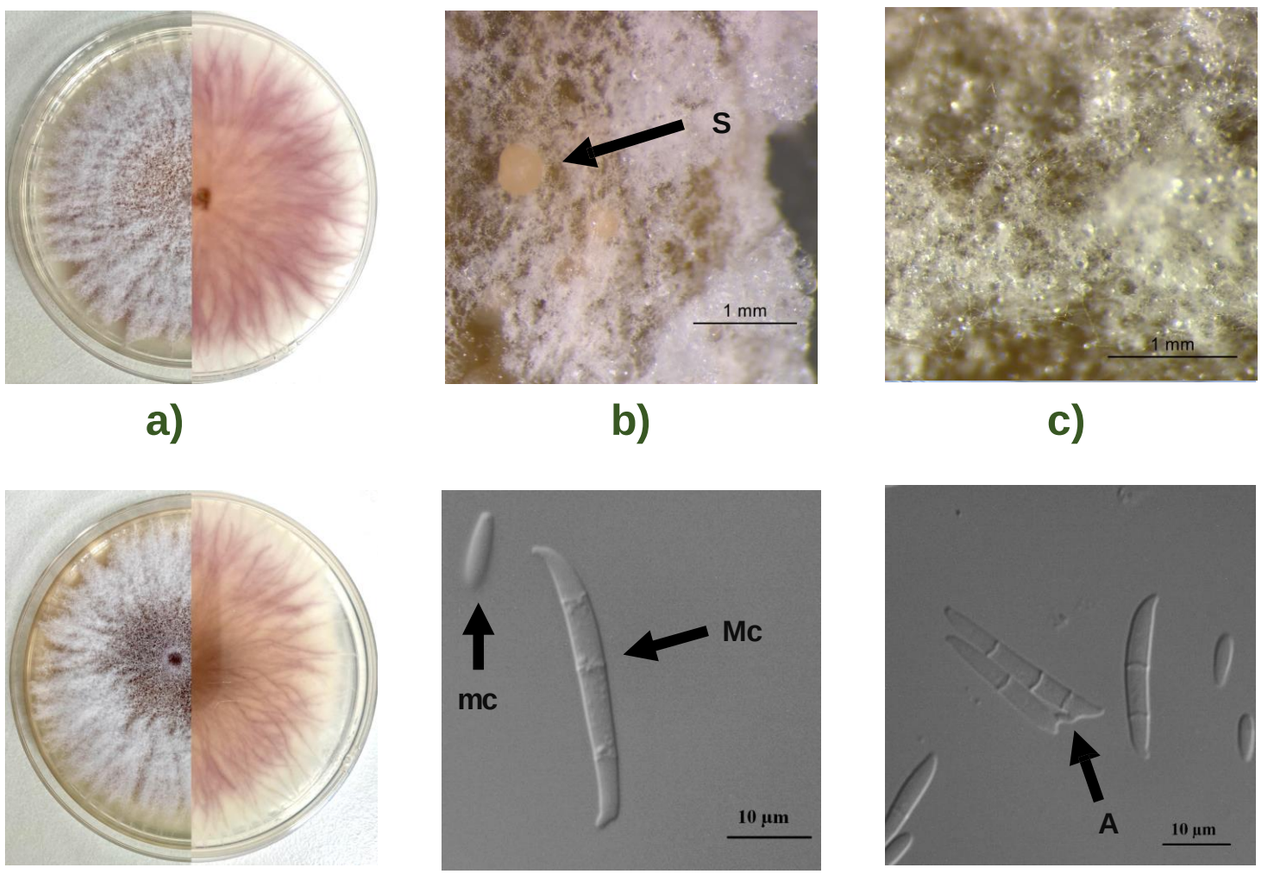
*Cymbopogon citratus* (DC.) Stapf (lemongrass) is a perennial widely distributed aromatic and medicinal plant of the Poaceae family rich in secondary metabolites. Most of *C. citratus* cultivation is related to essential oil (EO) extraction since their volatiles have applications in the food, fragrance, and pharmaceutical industries. This biological activity is greatly diversified, including bactericidal, insecticidal, fungicidal, and nematicidal activities. Thus, *C. citratus* EO can be a great source of new biochemical biopesticides, which can justify an increasing demand of this crop.

## Objectives

In the present study, the first report of *C. citratus* wilt caused by a FFSC species in Portugal is described. Symptomatologic, cultural, morphologic, genetic, and cytogenomic characteristics associated with this pathogen and disease are displayed. Ultimately, by examining these various aspects, this work aims to gain a comprehensive understanding of the wilt and its causal agent.

## Materials and Methods

1. Infected Plant Material and Isolation
2. Cultural and Morphological Caracterization
3. DNA Extraction, PCR Amplification and Sequencing
4. Cytogenomic Analysis with Flow Cytometry




a) b) c)

d) e) f)

**Figures 1:** Isolate growth on PDA after 7 days of incubation at 25 °C; (a) aerial and backplate view of a culture incubated with 12-hour cycle of light; (d) aerial and backplate view of a culture incubated in darkness. Sporodochia (S) formed in Carnation Leaf-Piece Agar (CLA), after 15 days of inoculation at 25 °C (b). Macroconidium (Mc) 3-septate and microconidium (mc) from sporodochia. (e) Mycelium of a colonized leaf at 25 °C (c). Anastomosis (A), a parasexual bridge, between macroconidia (f).

## Results and Discussion

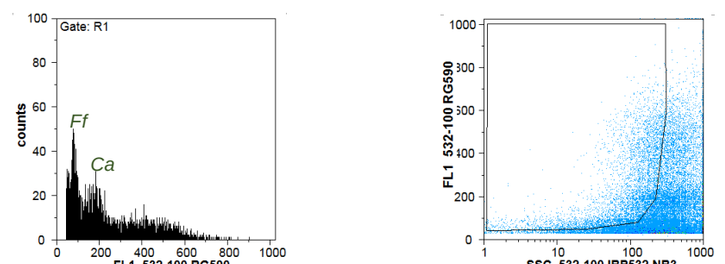
**Symptomatological Characteristics:** Leaf chlorosis followed by necrosis; collar rot and external fungal colonization; generalized wilt.

**Cultural Characteristics:** Filiforme margin and white colored colonies with abundant cottony aerial mycelia at the upper surface and orange-violet colored at the lower surface (Figures 1).

**Morphological Characteristics:** Septate macroconidia were formed in pale orange sporodochia present as discrete entities that be rare or obscured by thick mycelium. Macroconidia are fusoid with a flattened tapering toward the basal part and a number of septa ranging from 1 to 4. Microconidia were oval shaped with a flattened basal and 0-septate or rarely 1-septate. Chlamydospores were absent. A conidial anastomosis tube was present (Figures 1).

**Genetic Characteristics:** ITS region of ribosomal DNA region of the strain showed 100% similarity to *Fusarium fujikuroi* species complex.

**Cytogenomic Characteristics:** FFSC isolate has an estimate nuclear DNA content of about 0.0307 pg, which roughly corresponds to a genome size of 29.9 Mbp (Figures 2).




a) b)

**Figures 2:** Histogram of relative fluorescence intensities of propidium iodide-stained nuclei simultaneously isolated from mycelium of FFSC isolate (*Ff*) and the DNA reference standard, *Colletotrichum acutatum* (*Ca*) (**a**); dot-plot of side light scatter (SSC) vs. fluorescence pulse integral in linear scale applying a gating region to exclude as much as possible partial nuclei and other debris in order to improve the quality of the histogram (**b**).

Several diseases of *C. citratus* have a fungal aetiology, comprising species from several genera. Only *Fusarium equiseti* and *F. verticillium* have been associated with causing leaf spot and clump rot on *C. citratus* (Skaria et al., 2006). Neither of these species belongs to the FFSC, however collar rot and wilt of *Cymbopogon winterianus*, java citronella, have been associated with *Fusarium verticillioides*, a FFSC species (Alam et al., 1994). Nonetheless, microconidial chains, a key morphological feature of *F. verticillioides,* were absent. Futhermore, the cytogenomic data reveal a significant deviation of the genome size between the isolated fungus, 29.9 Mbp and *F. verticillioides*, 42.4 Mbp (Schoch et al., 2020). Considering these aspects jointly, it is clear that the isolate has a different aetiology from the previously reported FFSC pathogen of the *Cymbopogon* genus.

In future research, other isolated will be analysed and other genomic regions of this will be sequenced, such as TEF and RPB2 to determine the full phylogeny of this pathogen. Additionally, the Koch postulates must be entirely fulfilled to ensure the pathogenicity.

## Acknowledgments

The authors would like to thank Dr. Ana Cabral, Prof. Dr. Pedro Talhinhas, Prof. Dr. Helena Oliveira, and Prof. Dr. Maria Odete Torres, who in various ways, helped carry out this work.

## References

Alam, M., Chourasia, H. K., Sattar, A., & Janardhanan, K. K. (1994). Collar rot and wilt: a new disease of Java citronella (*Cymbopogon winterianus*) caused by *Fusarium moniliforme* Sheldon. *Plant pathology*, *43*(6), 1057-1061.

Schoch, C. L., Ciufo, S., Domrachev, M., Hotton, C. L., Kannan, S., Khovanskaya, R., ... & Karsch-Mizrachi, I. (2020). NCBI Taxonomy: a comprehensive update on curation, resources and tools. *Database*, *2020*.

Skaria, B. P., Joy, P. P., Mathew, S., & Mathew, G. (2006). Lemongrass. In *Handbook of herbs and spices* (pp. 400-419). Woodhead Publishing.