

Phylogenetic analysis of HCF164: Tracing S_H9-Mediated Coffee Leaf Rust Resistance and their conservation across Rubiaceae

Martim de Freitas^{1,2}, Carla Pinheiro^{2,3}, Leonor Guerra-Guimarães¹, Helena Azinheira¹, António Jesús Munoz-Pajares^{4,5}

Contact

hmga@edu.ulisboa.pt

¹ CIFC/LEAF/TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal; ² UCIBIO, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; ³ Associate Laboratory i4HB Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; ⁴ Departamento de Genética, Universidad de Granada, 18071 Granada, Spain; ⁵ Research Unit Modeling Nature, Universidad de Granada, 18071 Granada, Spain.

AIM

Coffee leaf rust (CLR), caused by *Hemileia vastatrix*, severely impacts *Coffea arabica* by reducing photosynthetic capacity and weakening plants. At least nine resistance factors (S_H1 to S_H9) have been identified in coffee, making the detection of genetic variants linked to resistance crucial for breeding and disease management [1]. Previous work suggests an association between the nuclear-encoded HCF164 chloroplast proteins with S_H9 phenotypes [2].

This work aims to conduct a phylogenetic analysis of *HCF164* gene variation across available Rubiaceae genomes.

APPROACH

Structural modeling reveals a unique HCF164 isoform in S_H9-resistant individuals, characterized by the loss of its thioredoxin domain (Fig. 1).

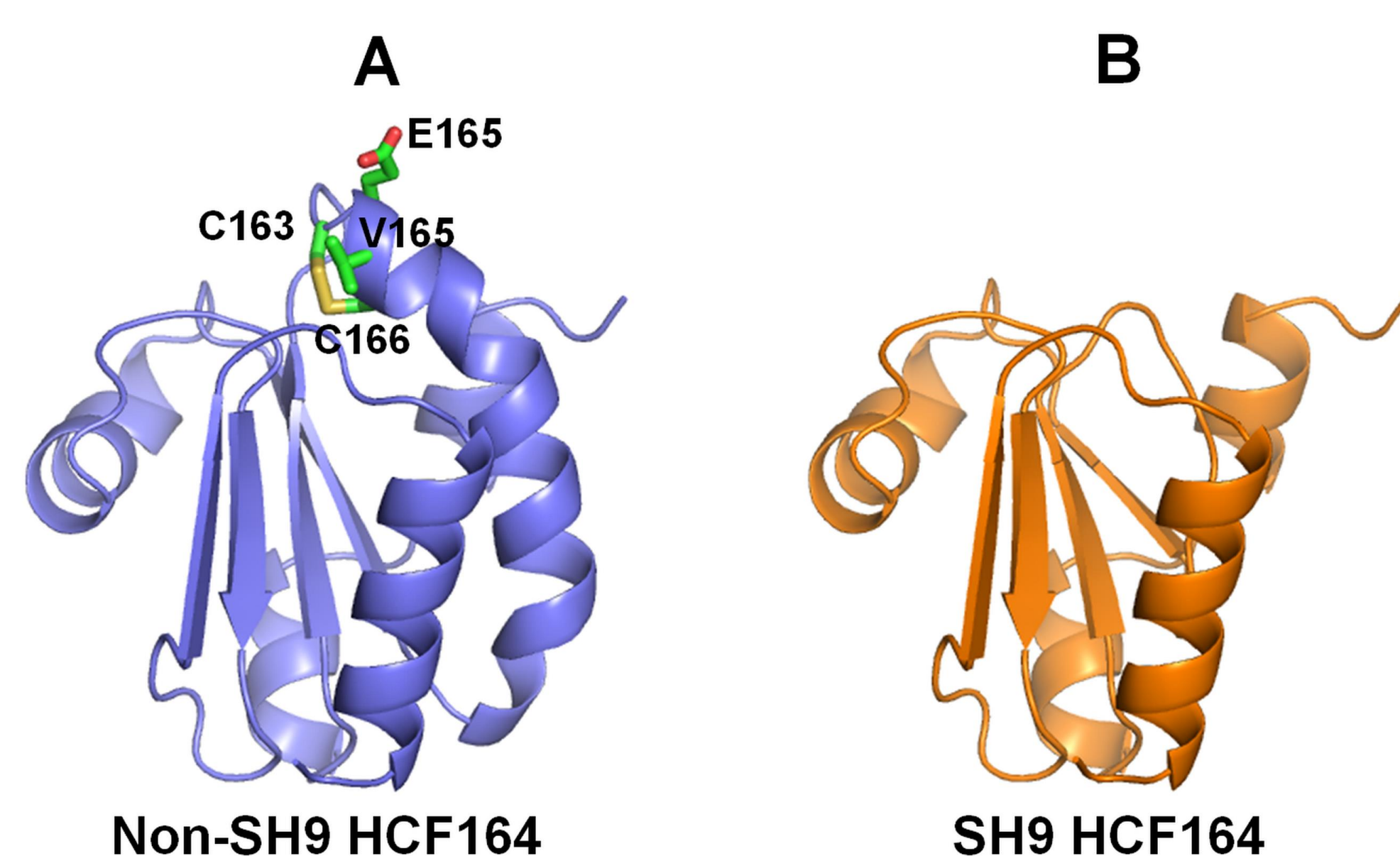


Figure 1 Models for HCF164 protein in non-S_H9 individuals (A) and S_H9 individuals (B). The CEVC residues forming the active center of the HCF164 protein in the non-S_H9 individuals are highlighted with green sticks, the α -helix containing the active center is not present in S_H9 individuals due to the 19-residue deletion [2].

Phylogenetic analysis of HCF164 DNA homologs was conducted on 60 Rubiaceae genotypes: 35 retrieved from NCBI RefSeq and 25 directly sequenced from biological material [2]. This resulted in 67 protein sequences, obtained either from NCBI RefSeq or predicted with AUGUSTUS.

All sequences were aligned with MAFFT and gaps coded with FastGap, and a Maximum Likelihood phylogeny was reconstructed using RAXML-NG. The HCF164 variant proteins, associated with S_H9 phenotype were mapped onto the phylogeny.

RESULTS AND DISCUSSION

The phylogenetic analysis of HCF164 protein sequences from all publicly available Rubiaceae genomes and from unpublished genomes generated within our team is presented as a cladogram (Fig. 2). Results revealed that HCF164 variants with active center deletions are unique to *Coffea* spp. Moreover, the findings confirm the association of this variant with the SH9-resistant phenotype, although SH factors have been identified for only 25 of the 42 previously analyzed genotypes.

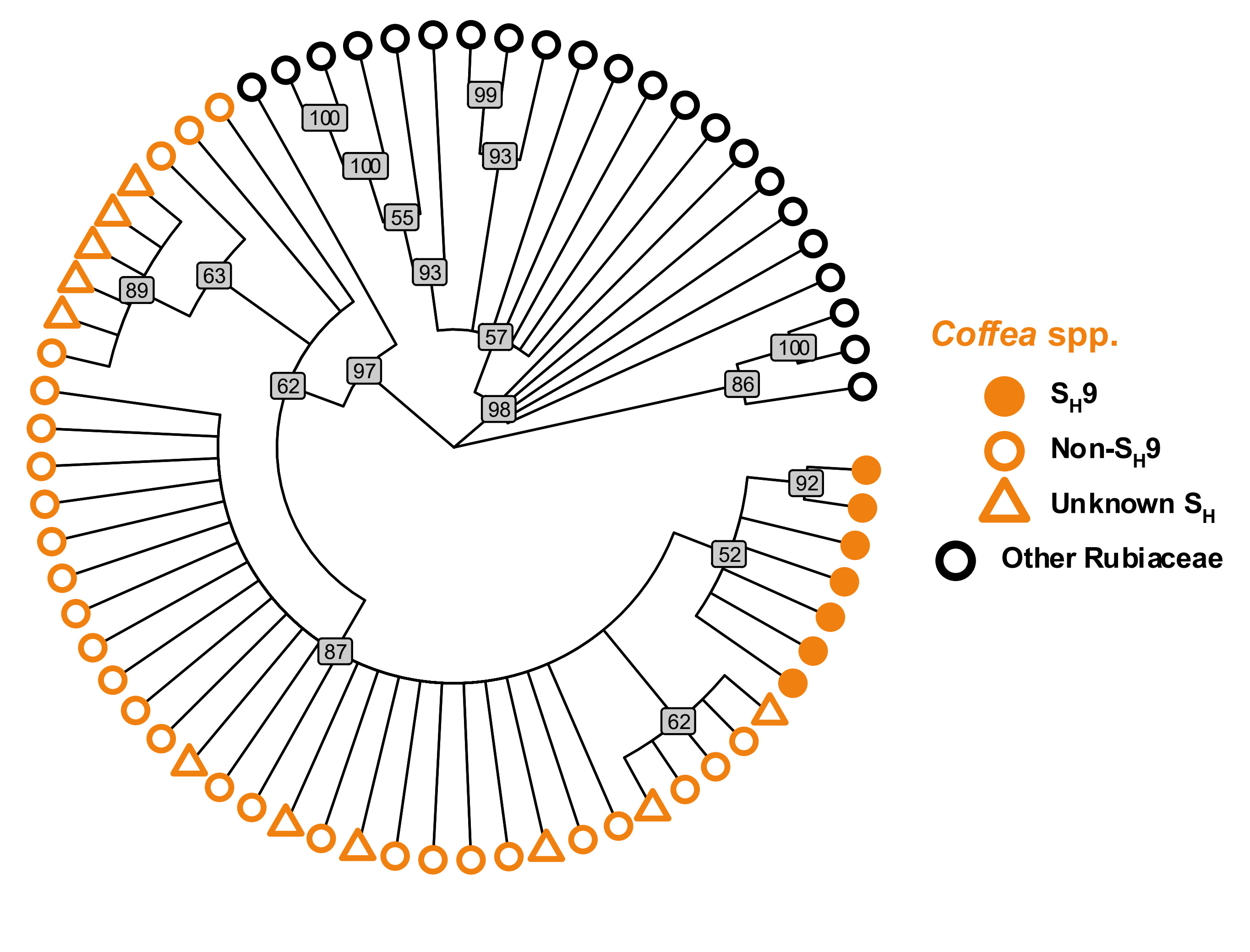


Figure 2: Cladogram of HCF164 protein sequences from various Rubiaceae species. Sequences retrieved from databases are marked as "Unknown S_H9". Bootstrap values are indicated inside the grey boxes.

CONCLUSION AND PERSPECTIVES

The phylogenetic analysis of HCF164 protein variants points to a novel origin within the Rubiaceae family. We aim to broaden this work to other plant families and to identify resistance-linked mutations.

This work will clarify HCF164's role in the rust resistance mechanism and validate its efficacy as a molecular marker for the S_H9 resistance factor.

[1] Silva MC. et al. Agronomy (2022) 12, 326

[2] Guerra-Guimarães et al. Sci. Reports (2023) 13, 16019