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Potential association of HCF164, a chloroplast nuclear-encoded thioredoxin-like protein, with *Coffea* S_H9 resistance factor against *Hemileia vastatrix*

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Rationale:
Coffee leaf rust, caused by *Hemileia vastatrix*, is one of the diseases most significantly affecting Arabica coffee production on a global scale. Previous studies of coffee-*H. vastatrix* interactions have identified nine coffee resistance factors, designated as S_H1 to S_H9. Considering the significance of primary carbon metabolism in plant fitness and coffee-*H. vastatrix* interactions, the chloroplast represents a prime target for pathogen manipulation. In this study we have performed whole genome sequencing of coffee genotypes to explore the connection between chloroplast and coffee resistance S_H factors.

Methods:
The chloroplast genome of 42 coffee genotypes from the CIFC collection with different resistance factors to *H. vastatrix* was sequenced and de novo assembled. A chloroplast phylogenetic haplotype network was performed. An *in-silico* analysis of 132 selected nuclear-encoded protein families acting on chloroplasts, focusing on gene families previously highlighted as being involved in *H. vastatrix* resistance, was also performed.

Results:
No maternal inheritance of coffee resistance factors throughout the chloroplast genome was evidenced. Indeed, the chloroplast phylogenetic haplotype network clustered individuals per species instead of per S_H factors. Nevertheless, it was possible to verify for the first time that *C. arabica* is the maternal parent of the Híbrido de Timor (HDT), a spontaneous hybrid between *C. arabica x C. canephora*. From all the 132 proteins analysed, only the thioredoxin-like membrane protein HCF164 was able to discriminate between individuals with and without the S_H9 factor. Thioredoxins are known to play crucial roles in redox regulation and defence mechanisms in plants and the lack of the thioredoxin domain and redox-active disulphide center in the HCF164 protein found in S_H9 individuals could potentially have functional implications.

Conclusions & Perspectives:
Our work reinforces the role of chloroplast-mediated defences against leaf rust and introduces an unexplored strategy for identifying protein/genes associated with S_H factors and candidate targets of *H. vastatrix* effectors, thereby creating new perspectives for coffee breeding programs.