Uncovering the genetic diversity of *Hemileia vastatrix* in three coffee-producing areas in Guatemala and its implications for resistance of coffee varieties

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Introduction

Coffee rust, caused by the fungus *Hemileia vastatrix*, is the leading disease that attacks the crop worldwide [1]. To control the disease, breeding of resistant coffee genotypes as well as understanding the molecular aspect of the pathogen [2]. This study assessed these aspects in *H. vastatrix* fungal populations across three coffee-producing departments in Guatemala with molecular markers along with investigating their correlation with the virulence level in eight coffee varieties.

Methodology

Sixty-four samples of *H. vastatrix* from 3 coffee-producing areas were fingerprinted with 17 SSR loci measured by fragment analysis. Data were analyzed by genetic diversity indexes (poppr [4]), clustering analysis (STRUCTURE [5] and Haplotype network [6]). For virulence assessment, uredospore solutions of three *H. vastatrix* haplotypes, representing the genetic groups, were prepared at a concentration of 10^6 uredospores/mL, and in vitro inoculations were performed on the leaf tissue of each coffee variety.

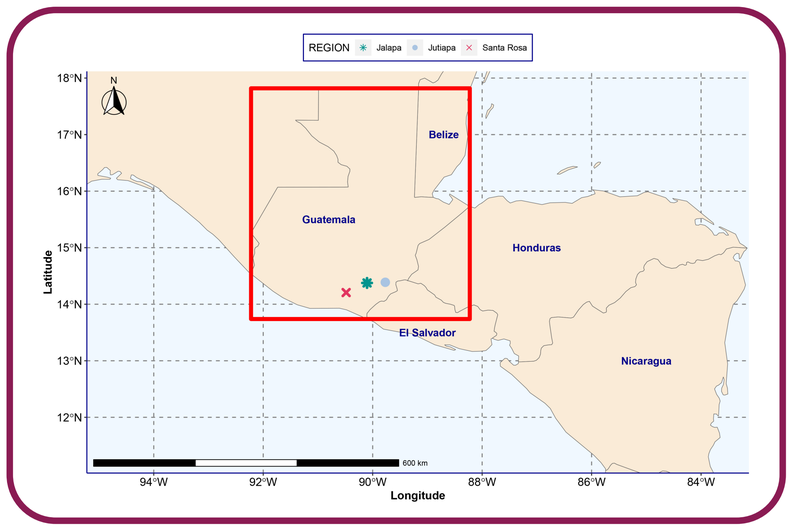

Figure 1. Map depicting the geographic distribution of *H. vastatrix* samples collected from three coffee-producing areas in Guatemala.

Results

Variation in expected heterozygosity (He) ranged from 0.47 in JAL01 to 0.69 in JUT01 (Table 1). AMOVA showed a variance between and within populations of 4% and 96%, respectively.

Table 1. Measures of genetic diversity of six populations of *H. vastatrix*.

Pop	N	Na	I	Ho	He	F
STA01	11	3.66	0.79	0.93	0.55	-0.6
STA02	09	5.25	0.85	0.92	0.59	-0.81
JUT01	13	3	1.11	0.93	0.69	-0.58
JUT02	11	4	0.86	0.91	0.54	-0.64
JAL01	07	4.33	0.75	0.81	0.47	-0.65
JAL02	13	4.1	0.74	0.97	0.61	-0.89
Mean	-	4.06	0.86	0.91	0.57	-0.68

Na = number of alleles; I = Shannon's information index; Ho = observed heterozygosity; He = expected heterozygosity; F = fixation index.

The population structure could be composed of three genetic clusters. However, a weak structure was observed (Fig 2A, 2B).

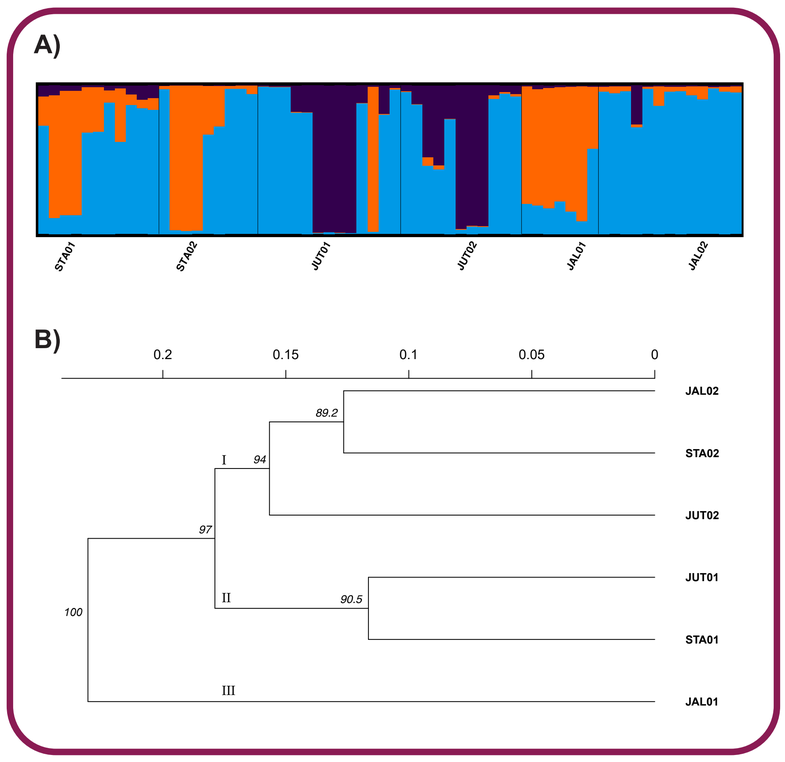

Figure 2. Population structure analysis of *H. vastatrix* using STRUCTURE (A) and UPGMA (B).

From the ITS region, 64 DNA sequences were obtained, resulting in 953 sites, of which 87 were variant sites and 22 were informative positions. 51 diverse haplotypes (Hs = 0.985) with low nucleotide diversity (Pi = 0.0051) were identified (Table 2).

Table 2. Variability Mesaures of *H. vastatrix* ITS Sequences from Guatemalan Samples

Pop	No. Sequences	Pi	H	Hs	D	SV	SPI
STA01	11	0.0044	10	0.98	-1.25	17	4
STA02	9	0.0057	8	0.97	-0.86	18	6
JUT01	13	0.0037	9	0.94	-0.58	13	6
JUT02	11	0.0071	10	0.98	-1.24	27	11
JAL01	7	0.0059	7	1	-1.31	18	2
JAL02	13	0.0043	11	0.97	-1.08	17	7
Total	64	0.0051	51	0.98	-2.53	87	22

Pi = nucleotide diversity; H = number of haplotypes; Hs = haplotype diversity; D = Tajima's test, SV = variable sites; SPI = informative parsimony sites

Haplotypes Hap_8 and Hap_09, considered ancestral, were widespread (Fig 3A). Phylogenetic analysis revealed two haplogroups, independent of geographic origins (Fig 3B).

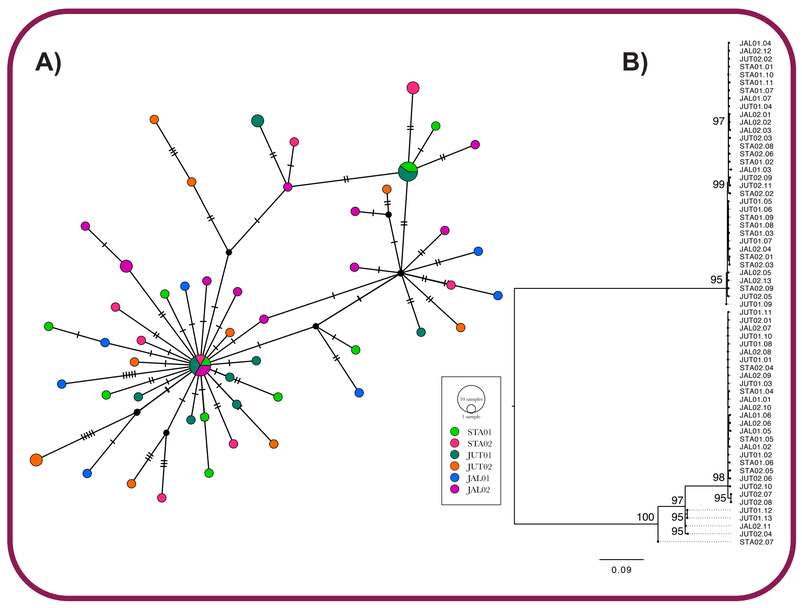

Figure 3. A) Haplotype network illustrating 51 unique ITS haplotypes of *H. vastatrix* from coffee-producing regions in Guatemala. B) Phylogenetic relationships among *H. vastatrix* ITS haplotypes with bootstrap values (>95) displayed.

Coffee varieties showed varying susceptibility to different *H. vastatrix* haplotypes. Significant differences ($p < 0.001$) were observed in AUDPC values for all variety*haplotype interactions. Hap_02 consistently had the highest impact, proving the most aggressive (Fig 4, Fig 5)

Figure 4. Inoculation of *H. vastatrix* haplotypes on coffee leaf tissue for virulence assessment.

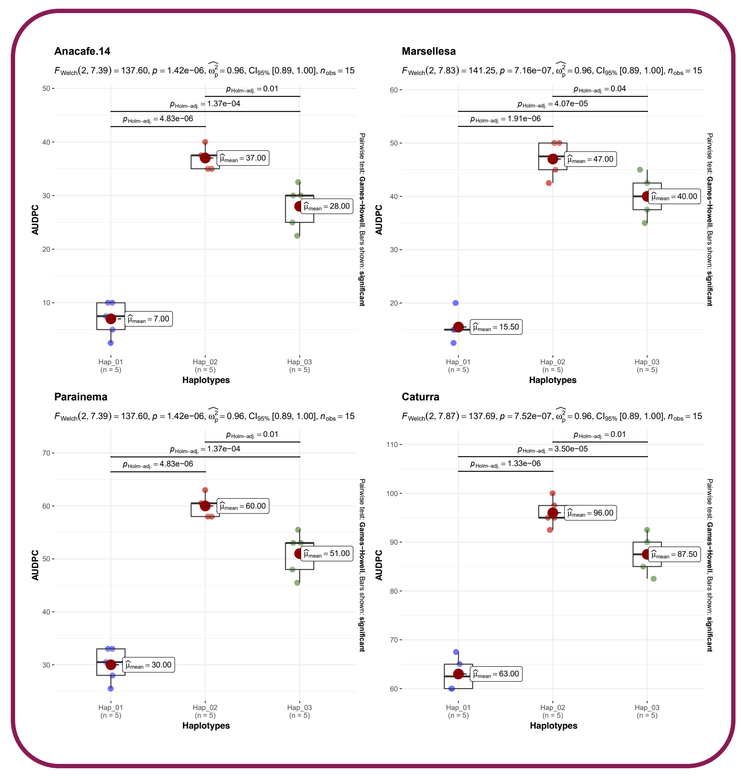

Figure 5. Box plot of AUDPC behavior in coffee varieties. It displays ANOVA results and only significant comparisons ($p < .05$).

Conclusions

In general, *H. vastatrix* populations in Guatemala are highly variable, and genetic variation is widely distributed in all the departments studied. It was determined that the haplotypic diversity of *H. vastatrix* may influence the resistance of coffee cultivars. Still, the interaction between the pathogen and the plant is complex, and other environmental factors may also influence resistance.

References

[1] P. Talhinhas *et al.*, *Mol. Plant Pathol.* **18**, 1039–1051 (2017). [2] M. D. Silva *et al.*, *Agronomy*. **12**, 326 (2022). [3] L. A. Ramírez *et al.*, *J. Fungi.* **8**, 189 (2022), doi:10.3390/jof8020189. [4] Z. Kamvar *et al.*, *PeerJ.* **2**, e281 (2014), doi:10.7717/peerj.281. [5] J. Pritchard *et al., Genetics* **155**, 945–959 (2000), doi:10.1111/j.1471-8286.2007.01758.x. [6] J. Leigh *et al*. *Methods Ecol. Evol.* 2015, **6**, 1110–1116 (2015), doi:https://doi.org/10.1111/2041-210X.12410.

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