

Novel insights from metagenomic data into population dynamics and lineage differentiation of the coffee leaf rust pathogen *Hemileia vastatrix* in Peru

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Introduction

Coffee leaf rust (CLR) caused by *Hemileia vastatrix* (Hv) has long gained a world-class status, reaching all coffee growing countries with major economic damages. Breeding for rust resistance has proven successful to control the disease, but the high adaptability of the fungus has shown to be a critical limitation for achieving durable CLR resistance. The last global epidemic that occurred mainly across the Americas was extremely severe in Peru, leading to a 60% decrease in harvest valued in US\$290 million, which motivated the implementation of a governmental emergency plan. In this context, understanding the genetic variation and structure of *H. vastatrix* populations across large geographic areas is a priority.

Here, we applied a targeted metagenomic approach to investigate population genetic diversity and differentiation, and evolutionary dynamics across the major Peruvian coffee production regions.

Sampling

Illumina sequence data of the ITS2 region was obtained from 70 *H. vastatrix* samples collected from 12 districts, distributed through the North, Center and South of Peru (55 field samples), and from a germoplasm bank (15 samples), comprising different coffee host genotypes.

Results

Sequence data analysis from the global sampling provided 8 OTUs and 22 ASVs. When comparing Hv populations from North, Center and South based on OTUs, no population differentiation was found with all haplotypes shared among samples (Fig. 2). In contrast, ASV analysis provided a clear clustering in two distinct genetic groups, differentiated by the presence of either ASV0 or ASV1, although with no apparent correlation with either with geography or host. This suggests a differentiation of two lineages (ASV0 and ASV1) from an ancestral haplotype (ASV2), which diverged independently in a high number of derived haplotypes matching the typical scenario of population expansion (Fig. 3 and 4).

OTU vs ASV dataset

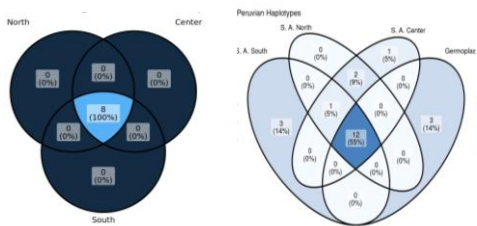


Fig.2. Venn diagrams of OTUs (A) and ASVs (B) shared among *Hv* population of North, Center, South regions of Peru and Germoplasm bank.

- ☐ Rust populations from **South** seem genetically **more diverse**, while the North populations harbour less diversity, which is reinforced by the detection of **3 exclusive haplotypes** in the South (2 from Huayopata and 1 from Maranura) and 1 haplotype in the Center (Perené).
- ☐ **Germoplasm** samples showed a much **higher diversity** than expected, with **3 exclusive and very divergent haplotypes**, which are not found in the field.
- ☐ Peruvian rust populations are structured into **two genetic lineages** evenly distributed across all coffee producing regions, which diverged independently from two main haplotypes. ASV1 lineage seems more prolific, which might entail a higher evolutionary potential

Conclusions and Perspectives

Our study provides a global high-resolution perspective on the genetic variation of *Hv* in the Peruvian coffee production regions, revealing for the first time a genetic structure differentiated into two lineages, although with no population geographical correlation. The southern population seems to have a higher degree of genetic diversity and differentiation, which may entail a different evolutionary potential.



Fig. 1. a) Coffee tree presenting severe defoliation resulting from CLR disease, b) and c) Abaxial side of coffee leaves exhibiting large orange colonies of uredospores on.

Data Analysis

Operational taxonomic unit (OTU) and Amplicon Sequence Variant (ASV) based analyses were performed with QIIME 1 and QIIME 2, respectively. Compositional heatmaps, alpha diversity measures, PCA and Parsimony Haplotype Network analyses were carried out.

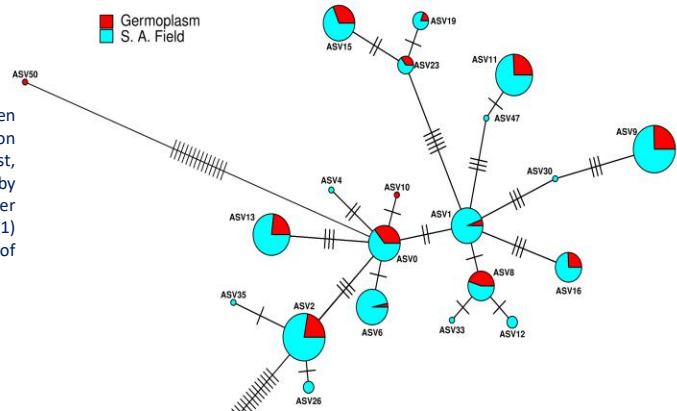


Fig.3. Haplotype network. Descendent haplotypes from lineages ASV0 and ASV1 differ by a small number of mutations.

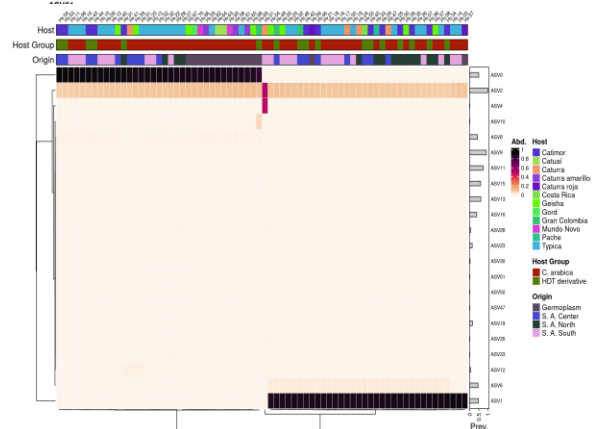


Fig.4. Composition heatmaps showing a clear structuring into two genetic groups, differentiated by the presence of either ASV0 or ASV1, while ASV2 is present in all samples. Within the two lineages, ASV8 appears only in samples with ASV0, while ASV6 appears only in samples with ASV1