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Assessing the involvement of retrotransposons in the genetic and genomic variability of *Hemileia vastatrix*

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Introduction

Hemileia vastatrix **(Hv) is an obligate biotrophic fungus causing Coffee Leaf Rust, the main disease of Arabica coffee, which leads to worldwide major production and financial losses. Since more than 50 Hv pathotypes have been described and considering the unknown host for Hv's sexual life cycle, it is essential to unveil the mechanisms promoting genetic variability and virulence in this pathogen in order to improve disease control. Retrotransposons have been implicated in genome shaping and expansion events, as well as in virulence increase [1]. In this study, we investigated the variation of Hv genome size, and the copy number and sequence polymorphism of 3 retrotransposons in different Hv isolates to address possible causal relations.**

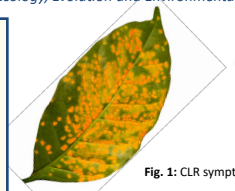

Fig. 1: CLR symptoms

Methodology

Annotated Hv EST Database [Talhinhas *et al*, 2014]

BLASTn

Diagnostic loci differentiating Hv genetic lineages [Silva *et al*., 2018]

Three candidate retrotransposons (RHv00190; RHv01057; RHv02407)

Estimation of genome size by flow citometry for 21 Hv isolates

Retrotransposon copy number estimation (qPCR) for 21 Hv isolates

Retrotransposon cloning (pGEM T-Easy Vector) and sequencing for 10 Hv isolates

Median-joining haplotype Networks (PopART v1.7)

Results

Table 1: Heatmap of genome size and retrotransposon's copy number per Hv isolate studied. Colour scale: Green to red correspond to increasing values (lowest to highest). NA: not available. Isolates in bold were used for the retrotransposon sequence variation study

Hv isolates	Genome size (Mbp)	No copies per genome		
		RHv00190	RHv01057	RHv02407
Hv3238	713	1112	NA	1143
Hv999	719	800	1	1216
Hv1321	721	1781	3	3377
Hv1427	751	1108	3	578
Hv741	758	873	4	1949
Hv999a	771	623	4	304
Hv178a	772	934	4	1008
Hv178	774	663	2	1109
Hv166	777	932	2	889
Hv1065	782	1650	103	1515
Hv995	783	725	3	1144
Hv264	788	1079	41	758
Hv3302	789	1435	6	649
Hv3305	792	3486	4	4457
Hv92	794	495	10	923
Hv71	824	1008	24	408
Hv264a	824	1771	39	728
Hv535	827	1934	2	1067
Hv22	834	725	NA	1322
Hv2191	842	1447	28	3460
Hv70	879	1848	2	1486

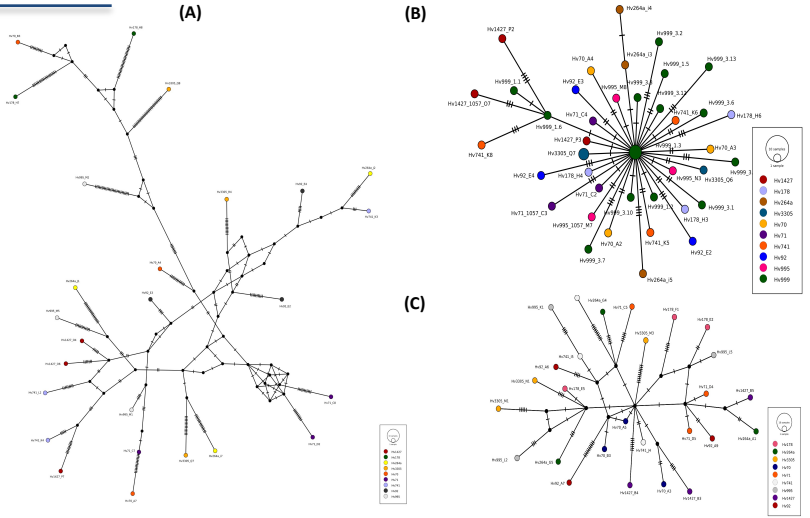

Fig. 2: Median-joining haplotype networks of 10 Hv isolates for the retrotransposons under study: **(A)** RHv00190 ; **(B)** RHv01057; **(C)** RHv02407

- ✓ **Flow cytometry data revealed high genome size variations among Hv isolates, which ranged between 713 and 879 Mbp, with an average size of 789 Mbp. Genome size differences between isolates could reach about 166 Mbp.**
- ✓ **qPCR analysis revealed a wide variation in retrotransposon copy number. RHv00190 and RHv02407 seem to be the most proliferative retrotransposons, ascending over 3000 copies per genome.**
- ✓ **No apparent correlation: bigger genomes do not imply higher number of retrotransposon copies.**

- ✓ **High levels of variability were found from sequence data analysis, revealing very divergent and singleton haplotypes/copies, corresponding to each sequenced *E. coli* colony, which could differ in more than 30 SNPs and up to 84 bp indels**
- ✓ **Haplotype networks revealed a complex diversification pattern, suggesting rapid evolution and proliferation, but no apparent relation with isolate/pathotype**
- ✓ **Higher proliferative retrotransposons have higher variability within copies**

Conclusions/Perspectives

Our study provides new data to potentially address coffee rust adaptive evolution and offers a first insight on the increased genetic variability provided by the presence of retrotransposons in the genome of Hv. In addition, although the differences in genomic content did not seem directly related with copy number for the retrotransposons under study, our results indicate a high level of proliferation and a possible active role of retrotransposons in Hv evolution.

References:
[1] Lorrain et al. (2021). DOI:10.1016/B978-0-12-819990-9.00042-1.
[2] Silva et al. (2018). DOI : 10.1111/mpp.12657.
[3] Talhinhas et al. (2014). DOI: 10.3389/fpls.2014.00088.

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