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Early Evidence for lncRNA Involvement in Coffee Leaf Rust Resistance

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AIM

Building on recent findings of differentially expressed long non-coding RNAs (lncRNAs) in the Kawisari hybrid during *Hemileia vastatrix* infection, this study aims to investigate their potential regulatory roles in coffee defense. As emerging modulators of gene expression, lncRNAs may contribute to coffee leaf rust (CLR) resistance by influencing proximal genes and shaping immune responses.

APPROACH

To validate the RNA-seq data generated in the CoffeeRES project and test the hypothesis that specific lncRNAs are involved in coffee resistance, we performed RT-qPCR to analyze the expression of selected lncRNAs and their flanking genes throughout the *H. vastatrix* infection process in Kawisari coffee leaves (Fig. 1). These expression profiles were subsequently integrated with structural predictions of the lncRNAs (Fig. 2).

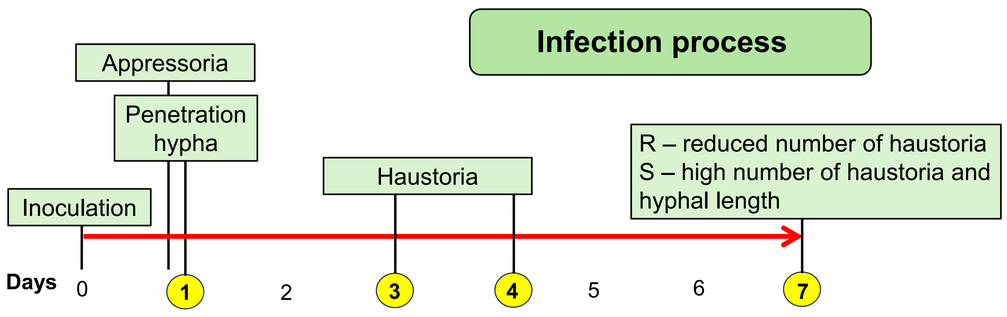

Fig. 1 – Timeline showing the main steps of the infection process of *H. vastatrix* on Kawisari coffee leaves, according to Diniz et al. 2021 [1]. Yellow circles indicate the time points of collected samples (R – Incompatible interaction, S – Compatible interaction).

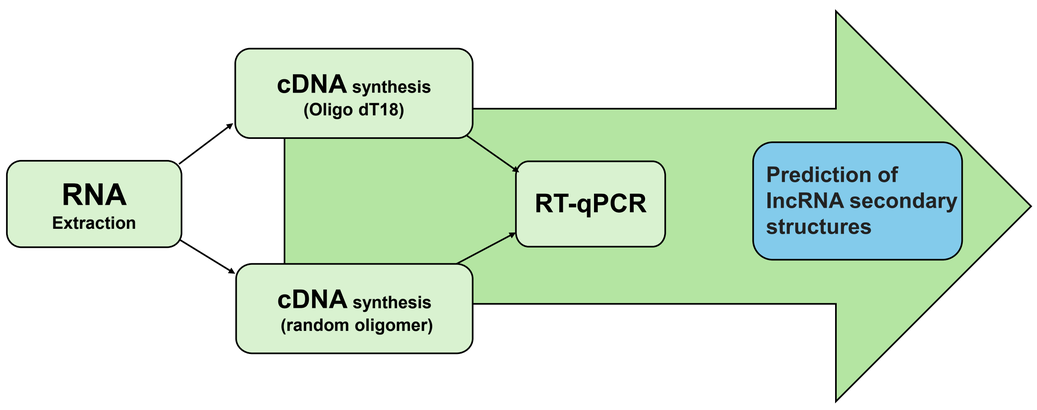

Fig. 2 – Protocol workflow

RESULTS

The lncRNA37 exhibited up-regulation in the resistant Kawisari cultivar, particularly by 4 and 7 days after inoculation (Fig.3A). Its predicted structure (Fig. 3B) showed a modular, multi-hairpin structure consistent with roles as a regulator in stress/immune pathways. It suggest its involvement in binding proteins or small RNAs, modulating defense gene expression, or acting as a structural switch during fungal infection process [2].

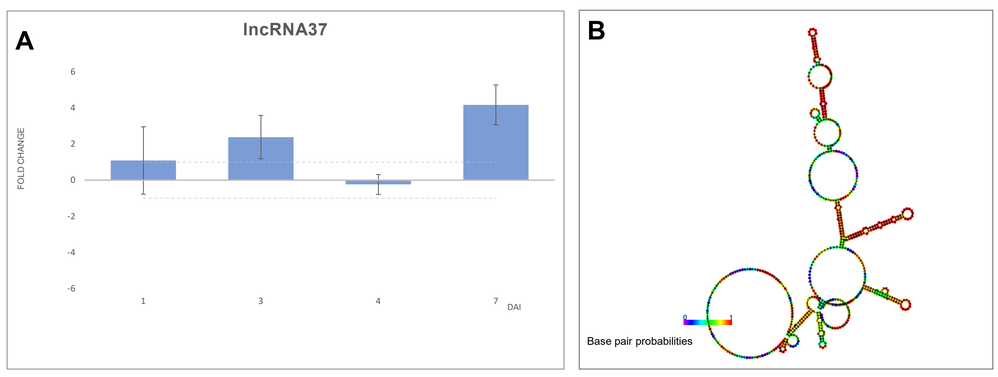

Fig. 3 – (A) Relative gene expression of lncRNA37 measured by RT-qPCR. Fold change was calculated using the Pfaffl method (2002) between resistant and susceptible samples at 1, 3, 4 and 7 days after inoculation (DAI). Actin and ubiquitin were used as reference genes. Data are presented as mean ± SD (n=3). **(B)** Predicted secondary structure of lncRNA37 presented with a color scale indicating the probability of the base pairs. The centroid structure was generated using the RNAfold WebServer (ViennaRNA Web Services - http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi).

Furthermore, the expression of lncRNA72 appears to correlate negatively with that of two neighbouring polyphenol oxidase (*ppo*) genes (Fig. 4). The structural features of lncRNA72 reveal a modular architecture combining stable and flexible domains, consistent with dual functions: providing scaffolds for protein complexes and serving as adapters for nucleic acid interactions. This combination of expression dynamics and structural versatility highlights its potential regulatory influence on neighbouring genes such as *ppo*.

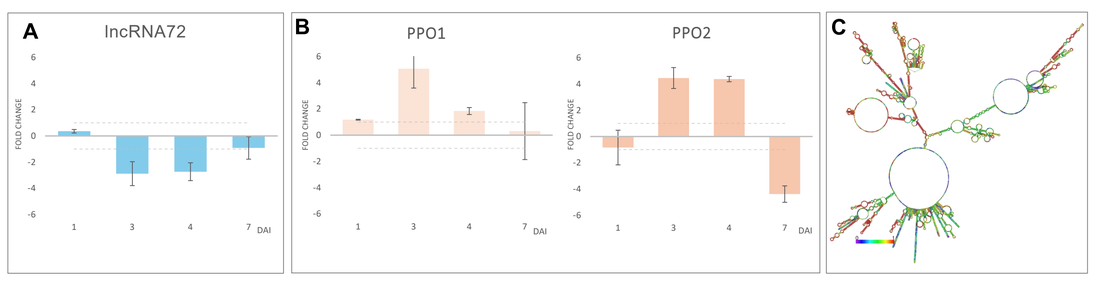

Fig. 4 – Relative gene expression of lncRNA72 **(A)** and the flanking *ppo* genes **(B)**, measured by RT-qPCR. Fold change was calculated using the Pfaffl method (2002) between resistant and susceptible samples at 1, 3, 4 and 7 days after inoculation (DAI). Actin and ubiquitin were used as reference genes. Data are presented as mean ± SD (n=3). **(C)** Predicted secondary structure of lncRNA72 presented with a color scale indicating the probability of the base pairs. The centroid structure was generated using the RNAfold WebServer (ViennaRNA Web Services).

CONCLUSION

The differential expression and structural flexibility of lncRNA72 and lncRNA37 suggest a putative role in dynamically regulating the coffee plant's response to CLR infection. To confirm this role and identify precise molecular targets, future work will employ functional validation through knockdown and overexpression assays. Ultimately, this research lays the foundation for leveraging lncRNA-mediated resistance in novel breeding strategies for durable CLR control.

ACKNOWLEDGEMENTS

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