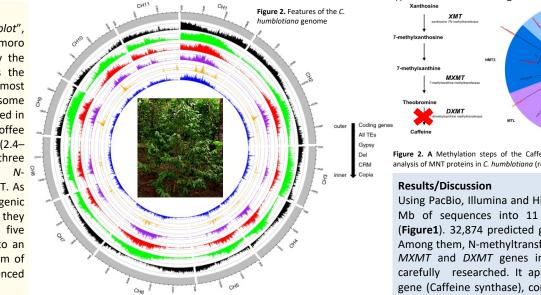
28th Conference The absence of the caffeine synthase gene is involved in the naturally decaffeinated status of *C*. S1-P-16 humblotiana, a wild species from Comoro archipelago

N. Raharimalala,¹ S. Rombauts,² A. McCarthy,³ A. Garavito,⁴ S. Orozco-Arias,⁵ L. Bellanger,⁶ A. Yadira Morales-Correa,⁴ S. Froger,⁶ S. Michaux,⁶ V. Berry,⁶ S. Metairon ⁶, C. Fournier⁶, M. Lepelley,⁷ L. Mueller,⁸ E. Couturon,⁹ P. Hamon,⁹ J.J. Rakotomalala,¹ P. Descombes,⁶ Romain Guyot,^{5,9} D. Crouzillat ⁷

¹ Centre National de Recherche Appliquée au Développement Rural, Madagascar; ² Department of Plant Biotechnology and Bioinformatics, Ghent University & VIB Center for Plant Systems Biology, 9052 Gent, Belgium; ³ European Molecular Biology Laboratory, Grenoble, France; ⁴ Departamento de Ciencias biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Caldas, Manizales, Colombia; ⁵ Universidad Autónoma de Manizales, Manizales, Colombia; ⁶ Nestle Research, Société des Produits Nestlé SA, Switzerland; ⁷ Nestle Research - Plant Science Research Unit, France; ⁸ Boyce Thompson Institute for Plant Research, Cornell University, USA; ⁹ IRD, UMR DIADE, France

Introduction

Coffea humblotiana Baill., also called "Caféier de Humblot", is the sole Coffea species endemic to the Comoro archipelago (Figure 1). It was probably consumed by the past. The remarkable feature of C. humblotiana, is the absence of caffeine in seeds and leaves, shared by most species from Madagascar, Mascarene Islands and some species from East and Central Africa. Caffeine is produced in the young leaves and immature fruits of African coffee species mainly, with a maximum level in C. canephora (2.4-3.3% dmb). The caffeine biosynthesis pathway involves three methylation steps catalyzed by different Nmethyltransferase genes (NMT): XMT, MXMT and DXMT. As output trait for the industry, caffeine as well as chlorogenic acid (CGA) compounds are of a great interest since they participate in producing metabolites which activates five human bitter taste receptors, therefore contributing to an inferior final cup quality. To understand the mechanism of the absence of caffeine in C. humblotiana, we sequenced and assembled the genome at the chromosome level.



Conclusion/Perspectives

The assembly of C. humblotiana provides the first high-quality reference genome for the Coffea genus. It provides valuable information for promoting its preservation and it represents a resource for genomic and evolutionary studies on Coffea. It is also of interest in helping to develop new strategies for characterizing coffee cup-quality traits. However, additional and substantial efforts will be necessary to develop strategies for improving coffee cultivars. In this regard, the absence of caffeine of C. humblotiana beans, that reduce the bitterness of coffee beverage, is an interesting avenue to follow.

Figure 2. A Methylation steps of the Caffeine biosynthesis. B. Phylogenetic analysis of MNT proteins in C. humblotiana (red) and C. canephora (black).

Using PacBio, Illumina and Hi-C, we assembled 422 Mb of sequences into 11 pseudochromosomes (Figure1). 32,874 predicted genes were identified. Among them. N-methyltransferases similar to XMT. MXMT and DXMT genes in C. canephora were carefully researched. It appears that the DXMT gene (Caffeine synthase), converting Theobromine to Caffeine, is absent from the C. humblotiana genome. Together with the biochemical absence of caffeine, low accumulation of Theobromine in leaves, and low expression of XMT and MXMT genes, we conclude to a probable regulation of the Caffeine pathway in C. humblotiana

References

Raharimalala, N., Rombauts, S., McCarthy, A. et al. The absence of the caffeine synthase gene is involved in the naturally decaffeinated status of Coffea humblotiana, a wild species from Comoro archipelago. Sci Rep 11, 8119 (2021). https://doi.org/10.1038/s41598-021-87419-0