

Compartmentalization of vanillin synthesis in *Phaeodactylum tricornutum*

Aracely Maribel Diaz-Garza¹, Brian Ingalls², Isabel Desgagné-Penix¹
¹Université du Québec à Trois-Rivières, Groupe de Recherche en Biologie Végétal.
²University of Waterloo, Department of Applied Mathematics.

Context

Vanilla flavor is composed by hundreds of compounds from which **vanillin** is responsible for most of the organoleptic characteristics of vanilla¹.

- *Vanilla planifolia* (fig. 1), the natural source, is a vining orchid which can only be cultivated in tropical areas².
- The extraction process from the vanilla beans possess a low yield³.
- Biotechnology is a promising alternative for **vanillin** production, since all the enzymes involved in its biosynthesis have been identified (fig. 2)²

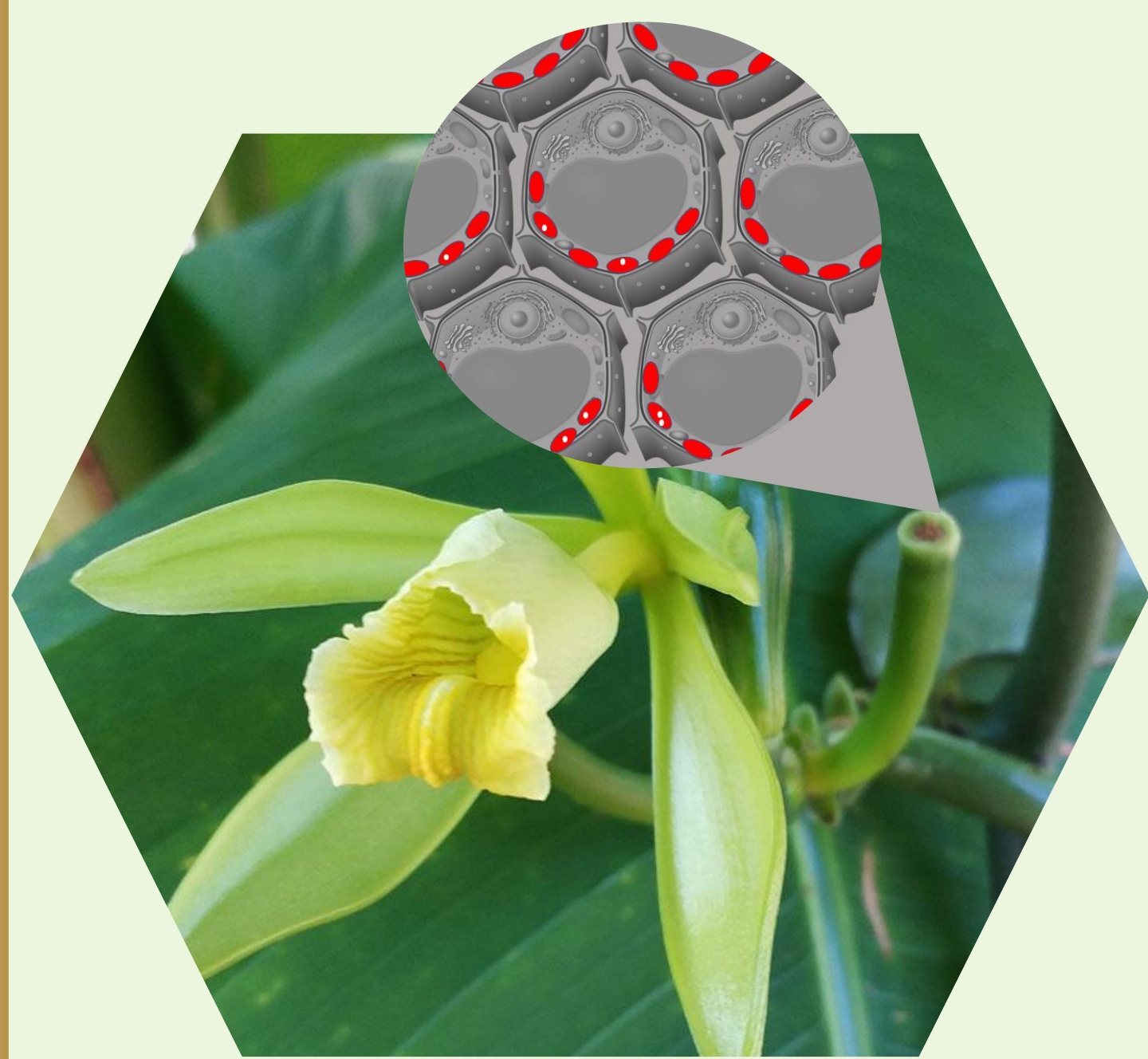


Fig 1. Scheme of VAN localized in the chloroplast of the *V. planifolia* pod. Chloroplast are represented in red, white spots simulate immunolocalization of VAN.

- Controversy on the last biosynthetic step catalyzed by *V. planifolia* vanillin synthase (**VpVAN**) which failed to produce vanillin conversion in various heterologous organisms⁴.
- Accumulation of VpVAN occurs naturally in a specific compartment of the plant cell, the chloroplast⁵.

Does VpVAN enzymatic activity depends on its localization in the chloroplast ?

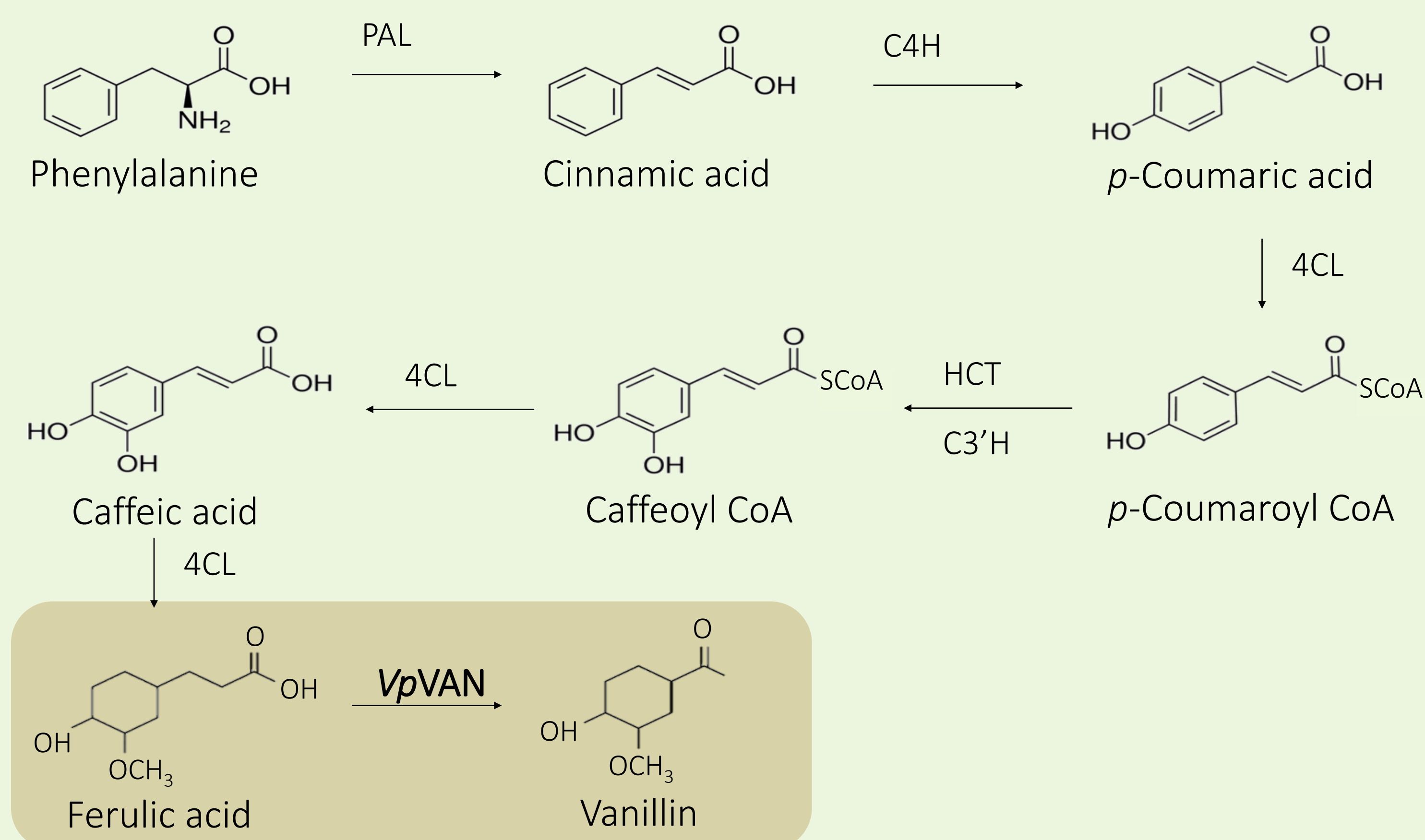
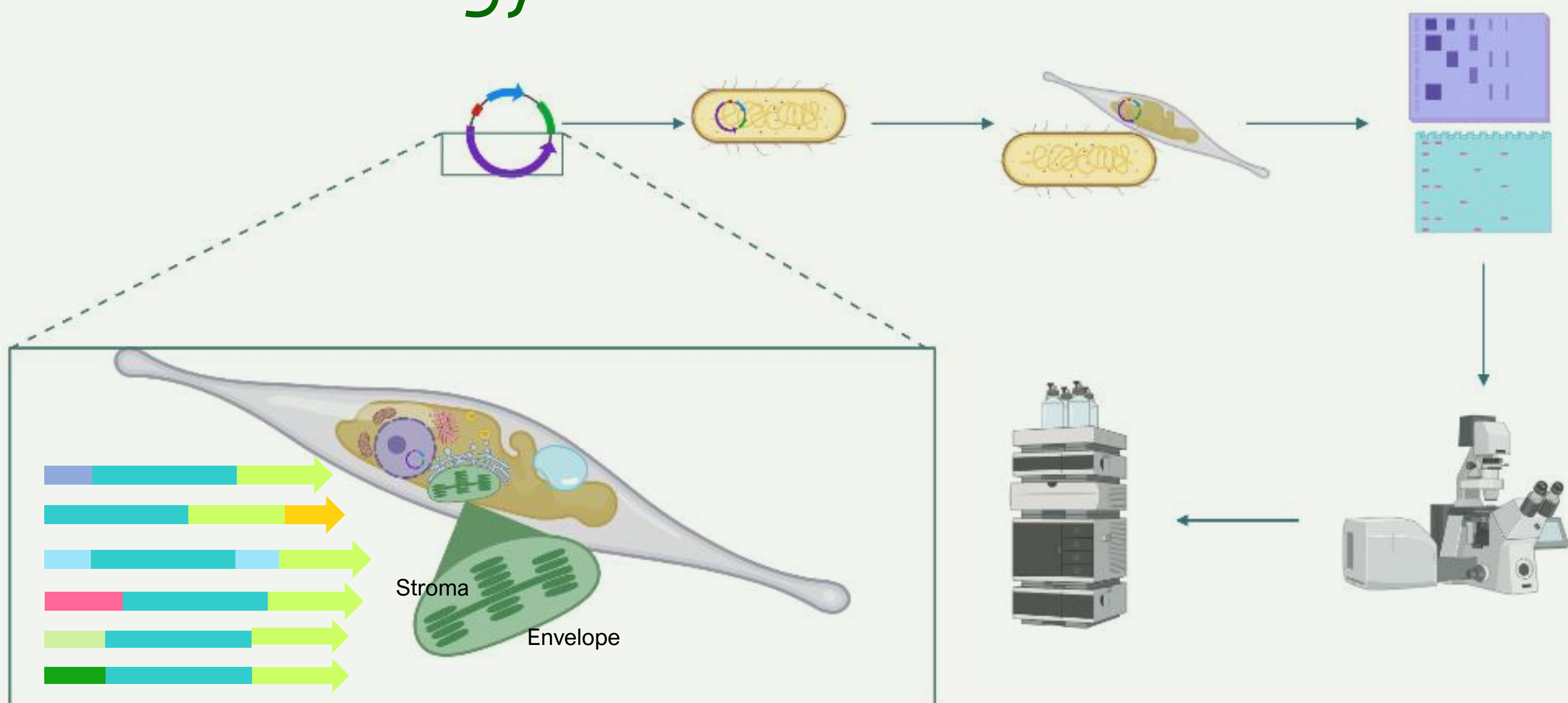


Fig 2. Proposed biosynthetic pathway of vanillin. VAN catalyzes the conversion of ferulic acid into vanillin.

Objective

Characterize the subcellular localization and enzymatic activity of VpVAN in the model diatom *P. tricornutum*.

Methodology



Results

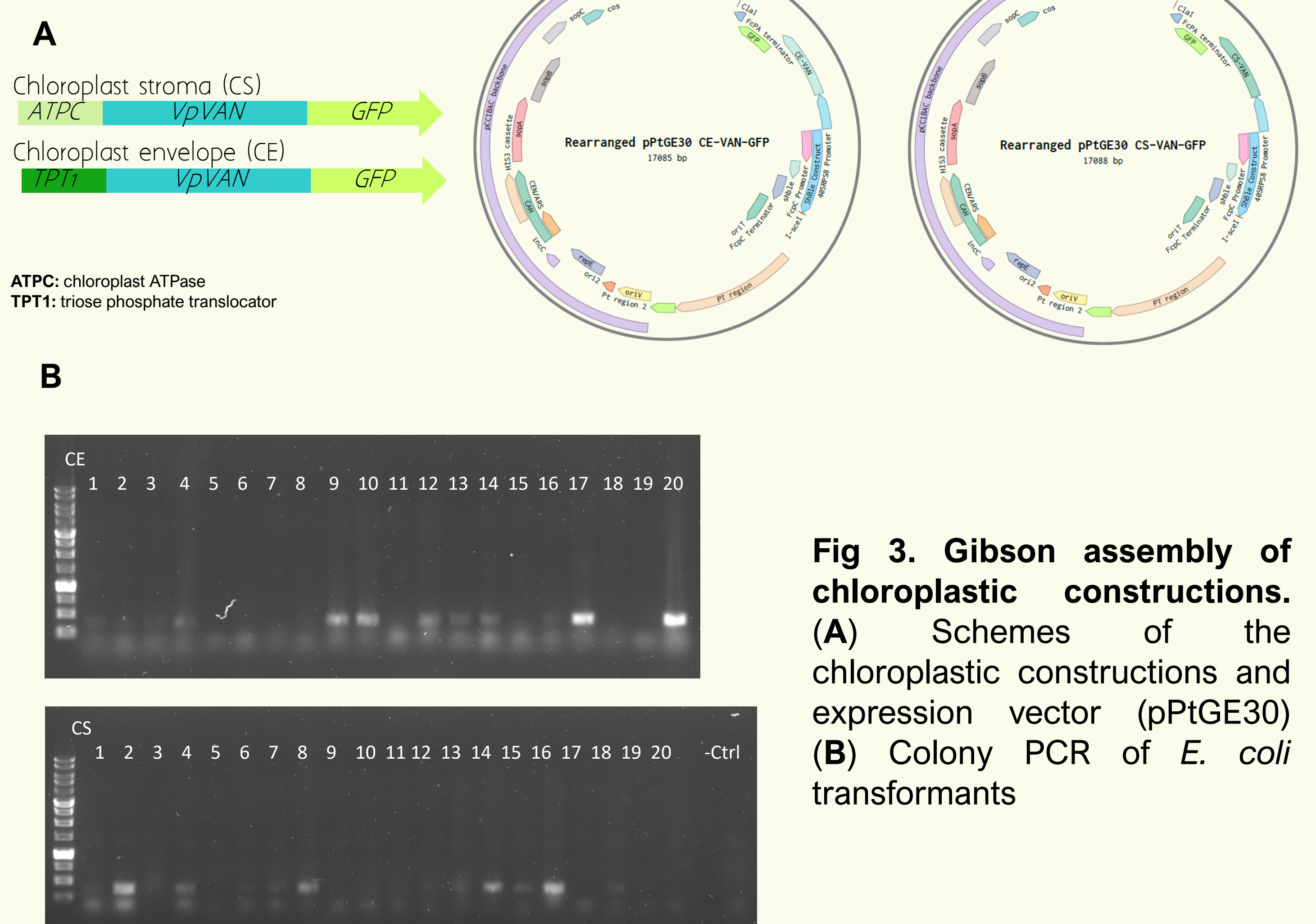


Fig 3. Gibson assembly of chloroplastic constructions. (A) Schemes of the chloroplastic constructions and expression vector (pPtGE30) (B) Colony PCR of *E. coli* transformants

Conclusions & perspectives

- Vectors with chloroplastic localization of VpVAN were successfully assembled and ready for transformation into *P. tricornutum*.
- Additional constructions will to be done to introduce VpVAN in various subcellular localizations (cytosolic, peroxisome, vacuole, Golgi, etc.) to evaluate its catalytic activity.
- This project has the potential of ending the controversy associated to vanillin synthase as well as contributing to develop a biotechnological process to synthesize vanillin.

References

1. Yan, L., Chen, P., Zhang, S., Li, S., Yan, X., Wang, N., Liang, N. & Li, H. Biotransformation of ferulic acid to vanillin in the packed bed-stirred fermentors. *Scientific Reports* 6, 34644 (2016). 2.
2. Hu, Y., Resende, M. F. R., Bombarely, A., Brym, M., Bassil, E. & Chambers, A. H. Genomics-based diversity analysis of Vanilla species using a Vanilla planifolia draft genome and Genotyping-By-Sequencing. *Sci Rep* 9, 1–16 (2019).
3. Gallage, N. J., Hansen, E. H., Kannangara, R., Olsen, C. E., Motawia, M. S., Jørgensen, K., Holme, I., Hebelstrup, K., Grisoni, M. & Møller, B. L. Vanillin formation from ferulic acid in Vanilla planifolia is catalysed by a single enzyme. *Nat Commun* 5, (2014).
4. Yang, H., Barros-Rios, J., Kourteva, G., Rao, X., Chen, F., Shen, H., Liu, C., Podstolski, A., Belanger, F., Havkin-Frenkel, D. & Dixon, R. A. A re-evaluation of the final step of vanillin biosynthesis in the orchid Vanilla planifolia. *Phytochemistry* 139, 33–46 (2017).
5. Gallage, N. J., Jørgensen, K., Janfelt, C., Nielsen, A. J. Z., Naake, T., Duński, E., Dalsten, L., Grisoni, M. & Møller, B. L. The Intracellular Localization of the Vanillin Biosynthetic Machinery in Pods of Vanilla planifolia. *Plant Cell Physiol* 59, 304–318 (2018).