IECPS 2024
The 3rd International Electronic Conference on Plant Sciences
15–17 January 2024 | Online

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# Optimization of enzymatic production in tamarillo (*Solanum betaceum* Cav.) cell suspension cultures using chemical elicitation

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## INTRODUCTION & AIM

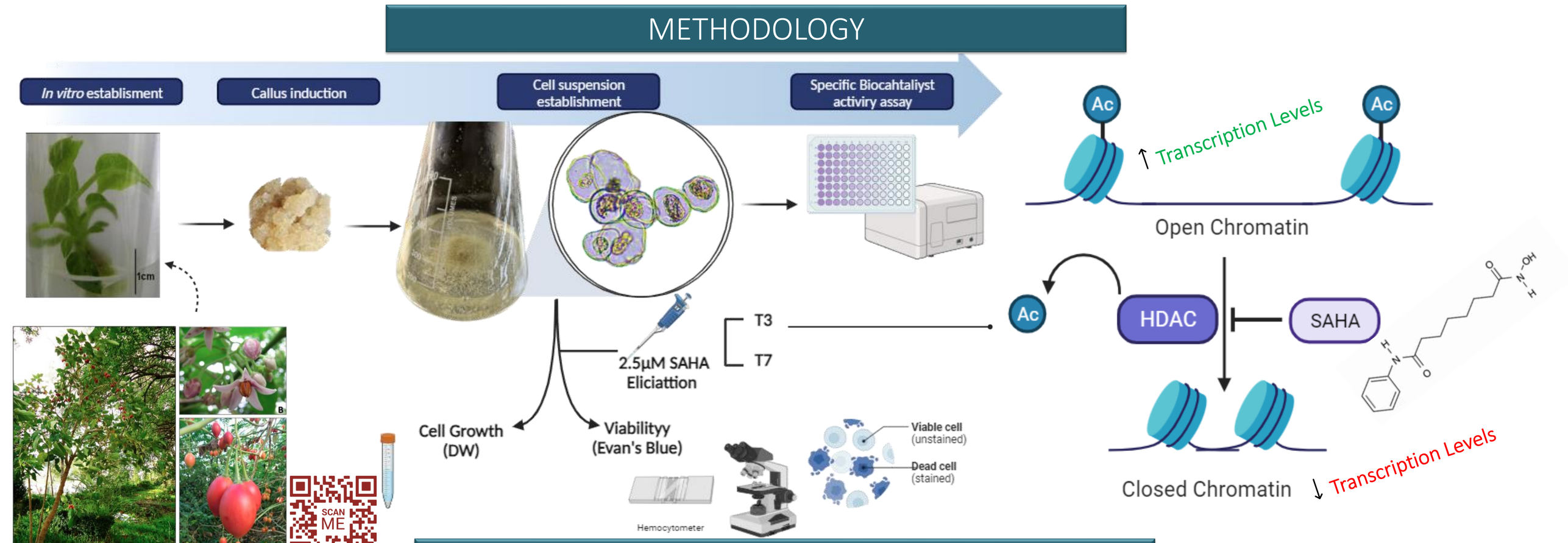
**Plant cell suspensions (PCS)** are sustainable and efficient systems for producing high-quality molecules within controlled bioreactors and contained environments, integrating **Molecular Farming** platforms.

**Histone acetylation,** performed by **HDAC's,** is linked to **heightened transcription levels**. Consequently, applying **HDAC inhibitors**, such as suberoylanilide hydroxamic acid **(SAHA)**, is anticipated to elevate mRNA and protein levels. In a previous study **[1]**, we successfully established **tamarillo-induced callus lines (ICL) PCS cultures**. Using various biotic elicitors, we induced the production of hydrolytic biocatalysts and low molecular weight peptides (>20 kDa), specifically **glycosidases, alkaline phosphatases, and proteases**, in tamarillo ICL PCS cultures.

We aimed to optimize the previously employed elicitation strategy **[1]**, testing the effect of the histone deacetylase inhibitor SAHA to **further enhance the production of hydrolytic biocatalysts**. The results demonstrated a significant **enhancement in specific biocatalyst production in SAHA-elicited tamarillo PCS cultures**, complementing the effects of previously used elicitors.

**We report** for the first time **the use of a histone deacetylase inhibitor as an elicitor for hydrolytic biocatalyst production in ICL PCS**, optimizing the elicitation strategy and contributing to overcoming the typical low-yield biocatalyst production of PCS. This advancement is a **crucial step forward in the potential scale-up of these systems to bioreactor production.**

## METHODOLOGY




## RESULTS

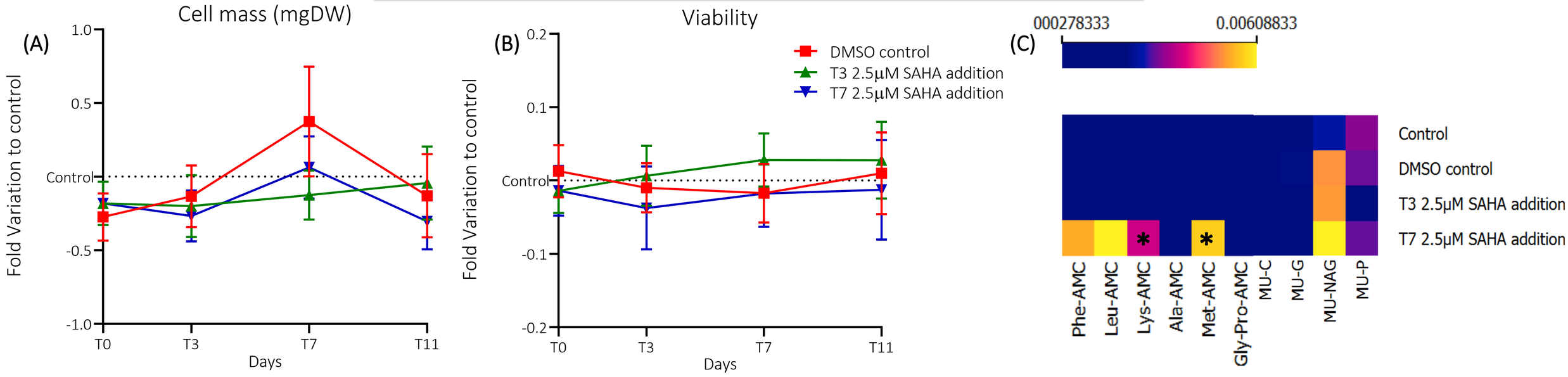



**Fig. 1- (A) Relative cell growth (DW)** and **(B) cell viability (Evan's Blue)** for 11-day period cultures, with **control** conditions**, DMSO addition and 2.5µM SAHA at T3 and T7** . **(C) Clustering** of **proteolytic profiles** of the assayed control and elicited cultures with and **2.5µM SAHA at T3 and T7**, using enzymatic activity assays. The first group of enzymatic substrates was used to evaluate the presence of **proteases** with a fluorogenic group in its C-terminal: amino methylcoumarine **(AMC)**. A second group of substrates was used to evaluate the presence of enzymes with the fluorogenic group methylumbelliferyl **(MU)**. **MU-G, MU-NAG and MU-C** are enzymatic substrates directed to **glycoside hydrolases and MU-P to alkaline phosphatases.** Data presented as mean ± SEM (n = 3). Statistical analysis with two-way analysis of variance (2wayANOVA) * $p < 0.05$).

## CONCLUSION

- **DMSO** didn't affected the **cell growth** and **cell viability**.
- **Cell growth and Viability** in line with control conditions, when SAHA is applied at **T3** or **T7**.
- **SAHA** at **2.5µM** applied on **T7** enhanced **proteases** activity **with statistical significance.**
- **SAHA** at **2.5µM** applied on **T7** enhanced **glycosidases** activity**.**

## ACKNOWLEDGEMENTS AND REFERENCES

This work was supported by the Foundation for Science and Technology (Portugal) through a doctoral research fellowship awarded to Bruno Casimiro (SFRH/BD/146485/2019) and the P2020|COMPETE grant number PTDC/BAA-AGR/32265/2017, BP4BP – Tamarillo breeding: better plants for better products, and the University of Coimbra / Santander Seed Projects Award 2021.

[1] B. Casimiro, I. Mota, P. Veríssimo, J. Canhoto, and S. Correia, "Enhancing the Production of Hydrolytic Enzymes in Elicited Tamarillo (*Solanum betaceum* Cav.) Cell Suspension Cultures," *Plants*, vol. 12, no. 1, Jan. 2023, doi: 10.3390/plants12010190.

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