Unraveling somatic embryogenesis signaling pathways – the role of extracellular molecules as efficiency modulators

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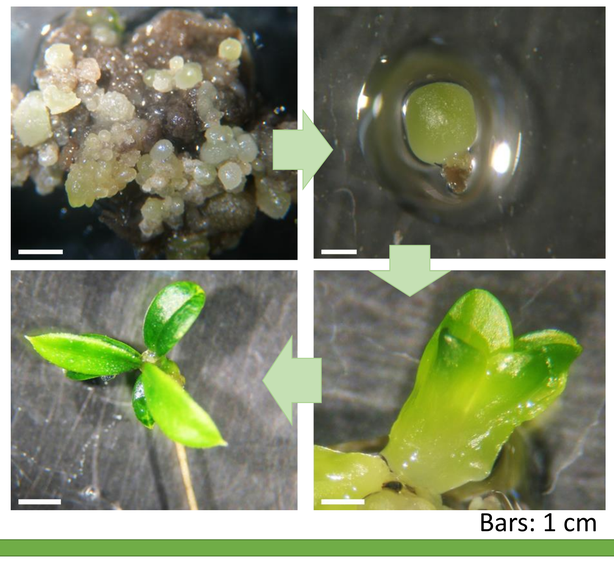
Summary

Somatic embryogenesis (SE) can be described as a process involving the formation of structures similar to the zygotic embryos, arising from dedifferentiation of somatic cells and not requiring the occurrence of fertilization.

This process, widely used in clonal propagation and genetic transformation of several plant species, is not routinely used in olive (*Olea europaea* sp. *europaea* L.), due to the recalcitrant behaviour that characterizes olive adult tissues.

An important factor referred as affecting SE in plants, never considered in any olive study, is the release of organic bioactive molecules by the explants into the culture medium.



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Extracellular molecules (RNAs, miRNAs, metabolites and proteins) are involved in several biological processes and can play an important role in cellular communications.

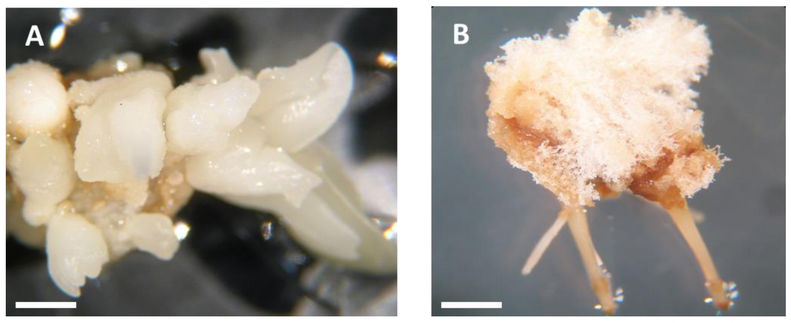
Based on this knowledge, a research line was established focused on the hypothesis:

"**Efficient SE lines secret to the extracellular environment biomolecules responsible for signalling that could be used to overcome recalcitrance**".

To explore this hypothesis three different omics will be considered: transcriptomics, proteomics and metabolomics.

Methodology

1. Establishment of olive somatic embryogenic cultures following a protocol already established [1].
2. Selection of two high efficient SE lines (Fig. A) and two low efficient SE lines (Fig. B).



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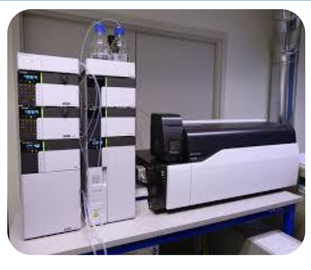
3. Characterization of the extracellular biomolecules following three different omics approaches.

Transcriptomics	Proteomics	Metabolomics
Sequencing of miRNAs	Secretome characterization	Metabolites Identification

Illumina (MiSeq)



Free label GC-MS/MS

RMN

4. The applicability of the extracellular biomolecules will be tested by establishing an *in vitro* assay using recalcitrant tissues as initial explants.

Preliminary Results

To test the applicability of the extracellular biomolecules, an *in vitro* assay will be established using recalcitrant tissues as initial explants treated with **liquid culture medium** provided from high efficient SE lines.

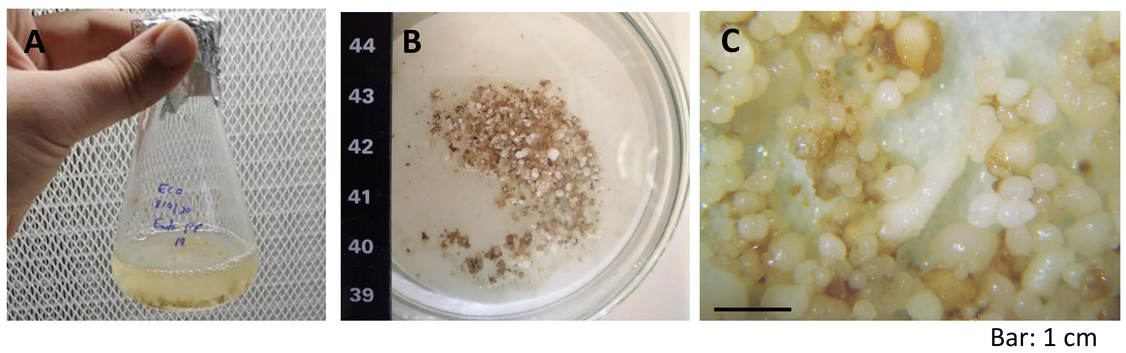
First experiments to establish the liquid cultures were already done:

High efficient olive somatic embryogenic cultures were established following protocol described by Pires et al (1).

Briefly, explants were grown individually on OMc induction medium (with 2.5 µM 2iP and 25 µM IBA) for 21 days, and further transferred to expression medium (OMc without growth regulators).

After the first embryos were formed, the calli were transferred to solid ECO (Olive cyclic embryogenesis) medium [2].

In the last phase, small fragments of calli were transferred to 100 ml erlenmeyers, containing 33 ml of liquid ECO culture medium with the same formulation (Fig. A).



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The number of embryos had increased significantly in liquid medium (Fig. B) and, in addition, the embryos also showed a more uniform appearance (Fig. C).

Final Considerations

The present work represent an important advance in scientific knowledge on fundamental topics such as the biochemical mechanism modulating the SE response associated to secreted proteins and/or metabolites. The use of the extracellular biomolecules is expected to achieve an innovative approach on enhancing SE potentiality in adult tissues of olive genotypes, particularly traditional Portuguese cultivars with high agronomic Interest.

Acknowledgments
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References
[1] Pires R, Cardoso H, Ribeiro A, Peixe A, Cordeiro A (2020) Plants. 758: 1-15.
[2] Cerezo S, Mercado JA, Pliego-Alfaro F (2011) Plant Cell Tissue Organ Cult. 106: 337-344.