Establishment of a somatic embryogenesis protocol for *in vitro* olive plant propagation – a focus on the valorization of the Portuguese cv. 'Galega vulgar'

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Summary

Due to its capacity to regenerate plants from a single cell, *in vitro* somatic embryogenesis, apart from being an efficient plant propagation system is also fundamental to assist plant breeding by genetic transformation, and fundamental research for validation of data coming from different omics. The establishment of efficient protocols for plant regeneration through somatic embryogenesis must be taken as the first achievement on the successful regeneration of a transgenic plant. In this way, a research work has been carried out to establish an efficient somatic embryogenesis protocol for the Portuguese olive cv. 'Galega vulgar' that could further assist research projects that follow a genetic transformation approach.

Material and Methods

For zygotic embryos it was followed the protocol of [1]. Seeds were removed from fruits (collected at the full ripeness stage) and further sterilized. Radicle, distal and proximal cotyledon regions were aseptically excised and inoculated in induction medium (with 2.5 µM 2iP and 25 µM IBA). After 21 days, explants with calli were transferred to expression medium (without growth regulators). To test adult material petioles taken from leaves of *in vitro* growing plantlets were used. To promote adventitious organogenesis, two different media were used: MS/2 (with 30 µM TDZ and 0.54 µM NAA) followed by OMs (with 10 µM 2iP, 2.2 µM BAP and hydrolysate casein) [2]. For repetitive somatic embryogenesis it was used the ECO medium following the protocol described by [3].

Results

Bar in all figures: 500 µm.

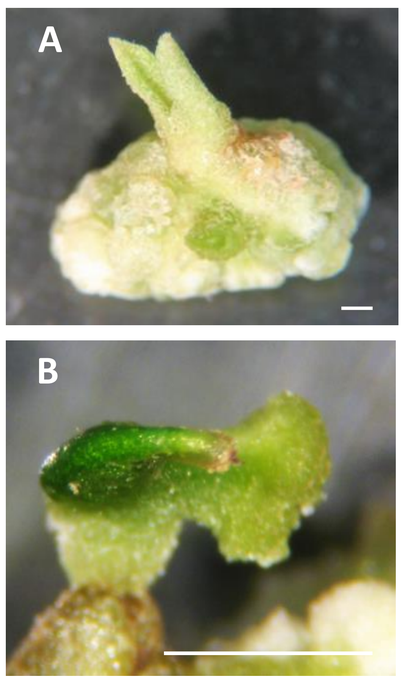

Figure 1. Leaf neoformations obtained from petiole..

The protocol followed for **adult material** required a pre-induction step allowing neoformation of adventitious leaves [2], further inoculated as explant to induce somatic embryogenesis.

The differentiation of adventitious leaves was achieved in both culture media tested, although with a better definition when explants were inoculated in MS/2 medium (Figure 1 – A) than in OMs medium (Figure 1 – B).

Success rates of pre-induction step was extremely low under both culture conditions, which, combined with contamination of cultures did not allow further testing, so it was not possible to test the conditions that could theoretically lead to somatic embryos.

In the **juvenile material**, the protocol suggested by [1] was fully established. Two photoperiod conditions (0 h and 16 h) were tested.

Significant differences were registered among explants used.

Radicles responded more efficiently to the embryogenic stimulus, exhibiting a higher number of differentiated somatic embryos, independently of photoperiod conditions.

Under dark conditions cotyledons were the most efficient explant (Figure 2).

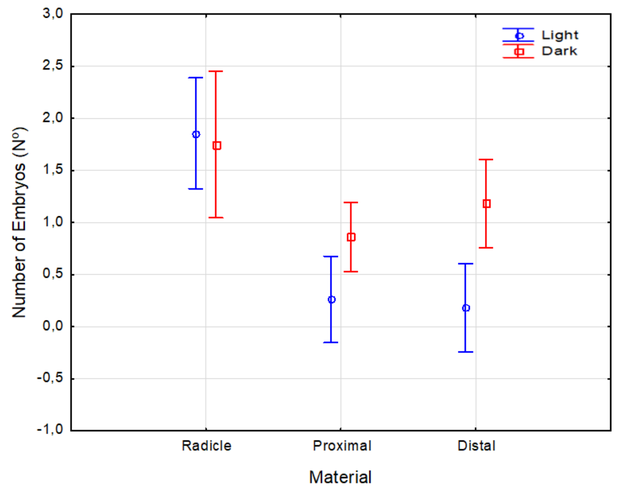

Figure 2. Number of embryos developed from three different explants (Material) under two photoperiod conditions (Light/ Dark – 16h/ 0h photoperiod). Vertical bars represent the 95% confidence intervals following a Fisher (LSD) test.

Repetitive somatic embryogenesis was achieved under light (Fig. 3 - A) and dark conditions (Fig. 3 - B). After calli inoculation in ECO medium [3] the number of embryos was significantly high than if compared with the number of embryos previously achieved in OMc medium without growth regulators, especially in light condition

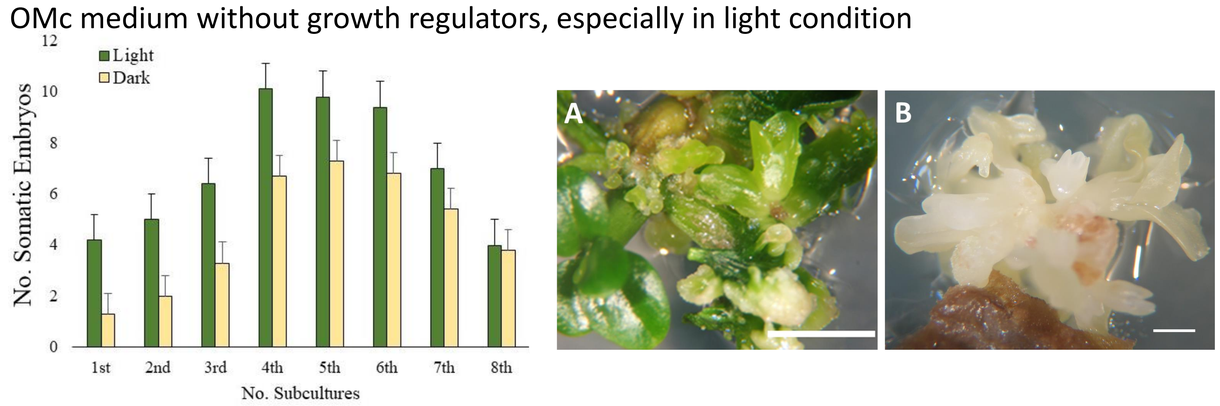

Figure 3. Repetitive embryogenesis in ECO culture medium. Radicle in light (A) and dark (B) condition. The chart represents the number of embryos during the various subcultures under the two conditions.

For **conversion and acclimatization** somatic embryos were removed from embryogenic calli and transferred to OMc solid culture medium devoid of growth regulators.

Shoots with 10 cm in height were transferred to polypropylene honeycomb trays with a substrate consisted on a mixture of sand, perlite and peat in the proportion of 1:1:3 (v/v).

At the end of the acclimatization period plants were transferred to a greenhouse where they remained 6 months (Fi. 4) to observe their development.

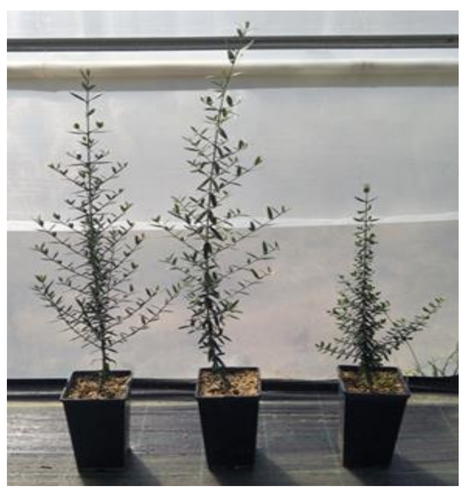

Figure 4. Plants originated from somatic embryos.

General Conclusions

In contrast to other cultivars that have been totally recalcitrant to this process, even in juvenile material, it was possible to obtain somatic embryogenesis in cv. 'Galega vulgar' when those materials were used. It was also noted that, in the adult material, in addition to a significant response of the explants in culture, regarding calli formation, it was also possible to obtain neoformation of leaf structures, a step considered essential to achieve somatic embryogenesis according to the adopted protocol. Considering the results achieved, it is possible to anticipate encouraging prospects for the establishment of a somatic embryogenesis protocol using adult material. However, it is necessary to continue the work started here, in order to understand the adaptations required for 'Galega vulgar', both at the explant level used, culture medium formulations and the physical conditions of the culture.

Acknowledgments

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