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Somatic Embryogenesis as a Tool for the Clonal Propagation of Elite Cork Oak Genotypes in Montado Restoration

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INTRODUCTION & AIM

Cork oaks (*Quercus suber*) are essential to the Montado ecosystem and regional economy, yet their survival is threatened by climate change and unsustainable practices. Repopulation with genetically superior, resilient trees is thus needed.

AIM: This research investigates the implementation of an innovative somatic embryogenesis (SE) protocol of cork oak on a unique F1 population with high genetic potential. The effects of genotype and explant developmental stage on the induction of SE were explored, together with osmotic stress during maturation phase, to develop a reliable clonal propagation method.

METHODOLOGY

A. Selection and plantation of *Quercus suber* individuals

1. Choice of initial progenitors

4 mother threes and 10 father trees from different Portugal regions were selected, based on quality cork and female flower productions.

2. Controlled pollination and germination of acorns

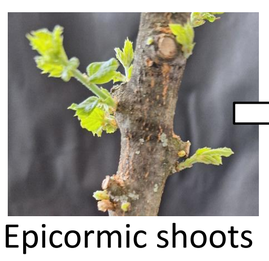
From 40 controlled pollinations using specialized bags, it resulted in 300 acorns, which were sown, and together formed 19 genetic families.

3. Plantation in the field

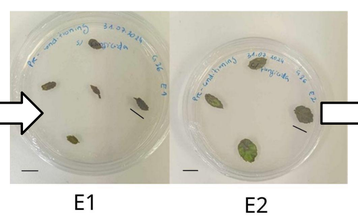
280 young *Q. suber* individuals were planted in Herdade da Abóbada, Serpa, Portugal.

B. Somatic embryogenesis of selected individuals

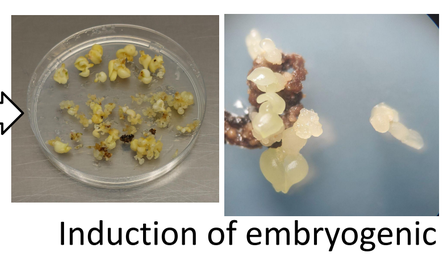
Three genotypes were selected for the SE: G31, G36, J2.



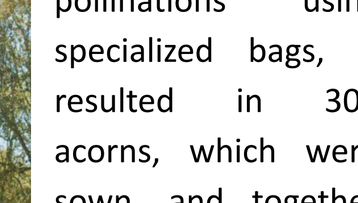
Epicormic shoots induction



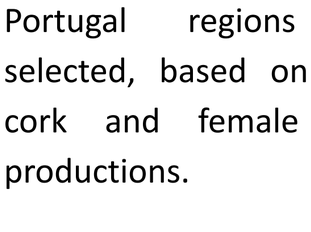
ES initial explant size: E1 (0.5-1mm), E2 (1-1.5mm). Bar=1cm



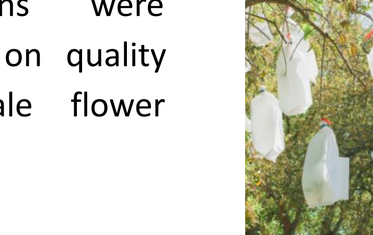
Induction of embryogenic *callus* on induction media[1]



Maturation in G31: control (expression media[1]) *vs.* osmotic stress (according to Sánchez H.[2]). Bar=0.5cm



Acclimation



Germination. Bar=0.5cm.

RESULTS & DISCUSSION

Both the induction of total *callus* and embryogenic *callus* were influenced significantly by **genotype** (Figures 1A and 1B). G31 hold the highest induction of embryogenic *callus* (70%, N=14), whilst J2 had 40% induction (N=12) and G36 none (N=15) (Figure 1B).

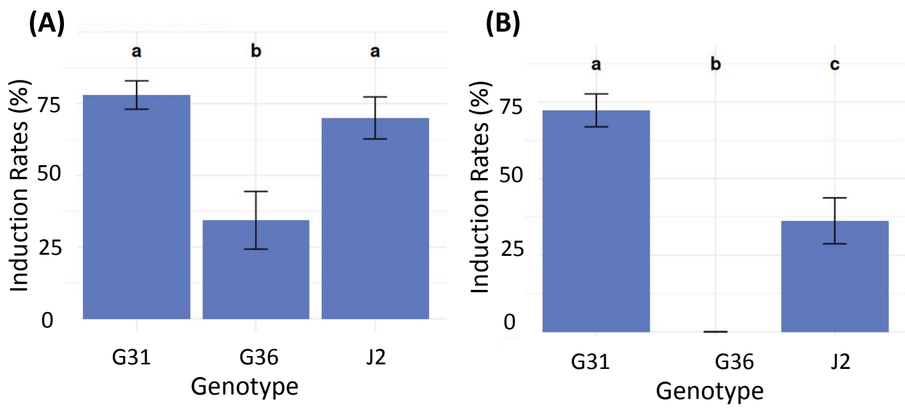


Figure 1: Induction rates of total callus (A) or embryogenic callus (B), per genotype. Mean±SE.
Stats: Kruskal wallis - Dunn's.

However, **initial explant developmental stage** (N=22 for E1, N=19 for E2) did not influence significantly induction rates (Figures 2A and 2B).

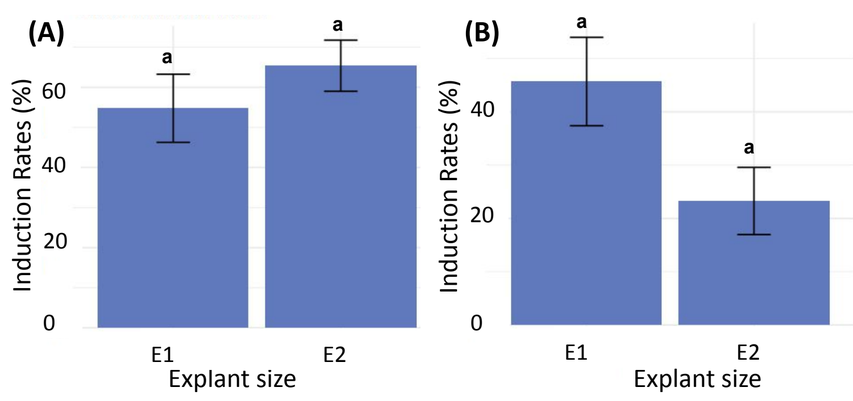


Figure 2: Induction rates of total callus (A) or embryogenic callus (B) per explant developmental stage (E1, E2). Mean±SE.
Stats: Wilcoxon rank sum test.

Inducing osmotic stress promoted the somatic maturation of the embryos when compared to control (Figures 3A and 3B, N=10 for each condition).

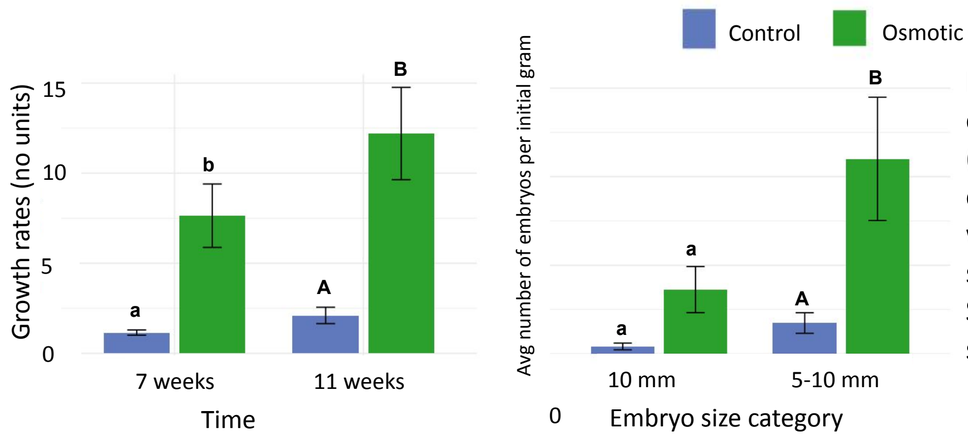


Figure 3: Growth rates of embryogenic masses (A) and average number of matured embryos with medium and large sizes (B).
Stats: Wilcoxon rank sum test.

CONCLUSION & FUTURE WORK

Different effects on both induction and maturation steps of SE were evaluated, being able to successfully inducing SE in two-thirds of the genotypes studied - genotype as the key factor - and most successful maturation by causing osmotic stress. Germination and acclimation remains a major hurdle, thus newer conditions and media are being tested, as well as other maturation media/conditions.

This work is a critical advance towards the clonal production of resilient trees needed to restore the threatened Montado ecosystem.

REFERENCES

1 - Hernández, I. *et al.* (2003), Plant Cell Reports, 21(8), pp. 765–770. doi:10.1007/s00299-003-0604-y

2- Sánchez, H. I. (2007). http://hdl.handle.net/10017/4608

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