EFFECT OF TYPE OF VESSEL AND SHAKING ON EMBRYOGENIC SUSPENSION CULTURES FROM A SELECTED CORK OAK TREE

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Breeding strategies based on Multivarietal Forestry can provide much more productive plantations.

Reliable and cost-effective methods of vegetative propagation are required to maximize genetic gains, by capturing all the genetic potential of selected individuals.

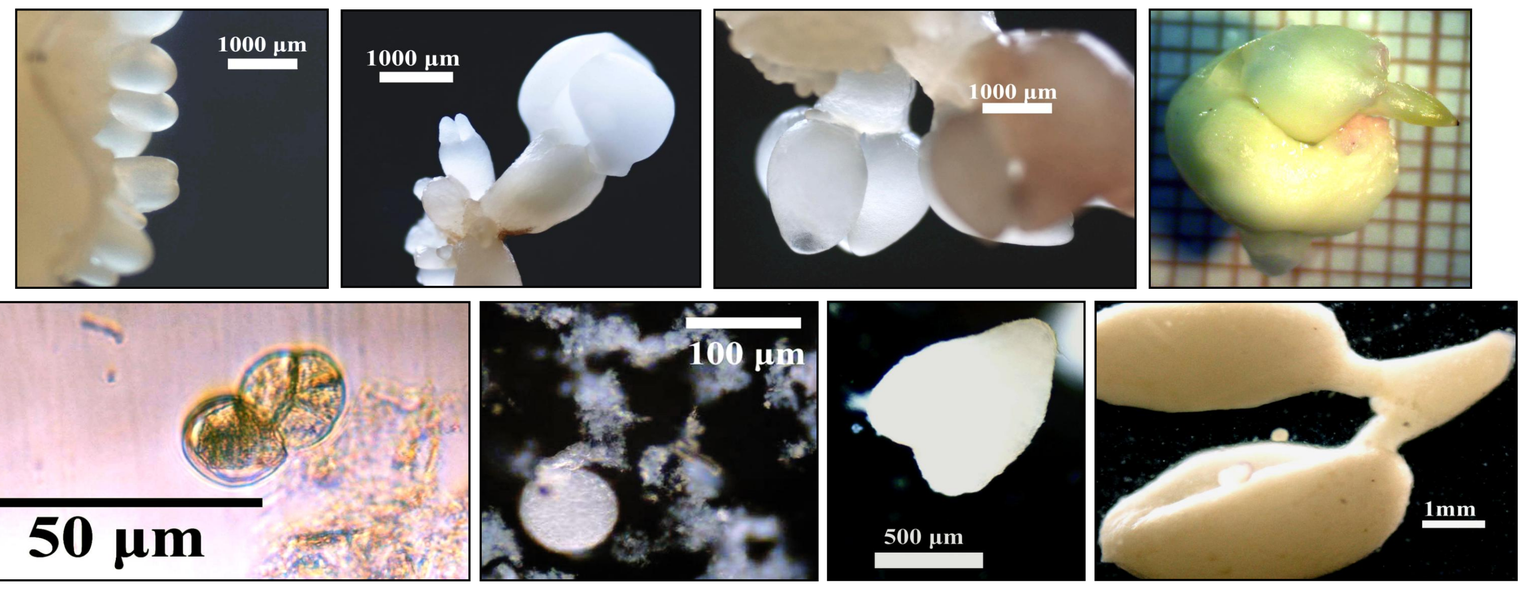
Somatic embryogenesis is increasingly used as the chosen method for mass cloning of forest species. We developed a protocol for plant regeneration of adult cork oak (*Quercus suber* L.) trees by somatic embryogenesis (Hernandez *et al*, 2003).

Secondary embryogenesis is the base of the large proliferation ability of this method. In cork oak cultured on semi-solid medium, secondary embryogenesis mainly follow a multicellular pathway of regeneration, although the unicellular origin was also evidenced (Puigderrajols *et al*, 1996).

For low-cost mass production of clonal plants, culture in liquid medium in bioreactors is required. We established suspension cultures of embryogenic tissues cork oak (Jiménez *et al*, 2007) and the complete formation of isolated somatic embryos of putative unicellular origin has been found.

EXPERIMENTAL

Objective: Effect of vessel and shaking on embryogenic tissue production and on some culture medium parameters.

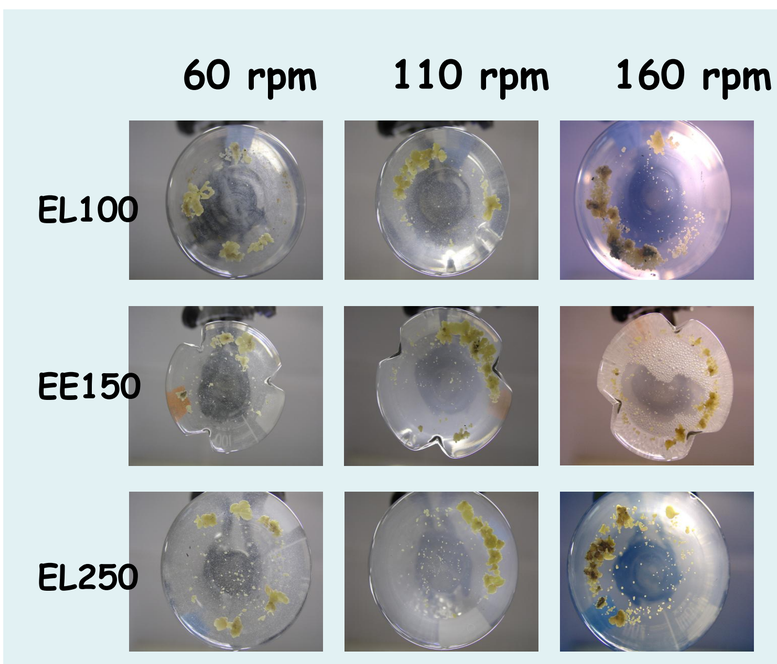


Material & Methods
Genotype ALM80. established in liquid culture.
Initial inoculum: four embryogenic aggregates of 800-1200 µm (0.14-0.21 mg) in 70 ml of MS medium with SH macronutrients per vessel. Density: 2-3 mg/l.
Complete factorial design.
Culture period: six weeks.

Analysis of variance of the effect of type of vessel and orbiting speed in the shaking flasks culture system on the produced biomass, the number and frequency of formed embryogenic clusters, and on the pH, the electric conductivity (EC) and the dissolved oxygen (DO), at the end of the six-week culture period. Mean values from ANOVA and statistical significance of each factor

Factor	Biomass	Number of aggregates				Frequency (%)					
	mg FW	Total	Big	Medium	Small	Big	Medium	Small	pH	EC	DO
Type of vessel											
EF_{100}	524	1105	181	181	743	16.4	14.0	69.6	5.4	2.9	56.6
BEF_{150}	568	1789	256	326	1206	14.7	16.8	68.6	5.6	2.8	64.0
EF_{250}	508	1305	140	224	941	9.7	16.1	74.2	5.9	2.7	66.4
Orbiting speed (rpm)											
60	372	932	103	73	756	10.5	6.7	82.8	5.3	3.0	64.7
110	564	1051	177	218	655	17.1	20.4	62.5	5.9	2.6	60.6
160	664	2217	299	439	1479	13.2	19.8	67.0	5.8	2.8	61.7
Overall mean	533	1400	193	244	963	13.6	15.6	70.8	5.7	2.8	62.3
Significance of factor											
Type of vessel	0.752	0.000	0.015	0.011	0.000	0.010	0.524	0.342	0.002	0.051	0.004
Orbiting speed	0.006	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.001	0.000	0.282
Vessel × Speed	0.129	0.006	0.146	0.080	0.001	0.034	0.349	0.434	0.572	0.170	0.507

EF100: Erlenmeyer flask 100 ml; BEF150: Baffled Erlenmeyer flask 150 ml; EF250: Erlenmeyer flask 250 ml
Big aggregates (> 800 µm); medium aggregates (800 – 180 µm); small aggregates (180 - 40 µm)



Hernández I, Celestino C, Alegre J, Toribio M (2003) Vegetative propagation of *Quercus suber* L. by somatic embriogénesis. II. Plant regeneration from selected cork oak trees. *Plant Cell Rep* 21:765-770.
Jiménez J, López-Vela D, Hernández I, Carneros E, Celestino C, Toribio M, Alegre J. (2007) Iniciación del cultivo de embriones de alcornoque (*Quercus suber* L.) en medio líquido: caracterización de suspensiones obtenidas en función del tiempo en cultivo. VII Reunión Sociedad Española Cultivo *in vitro* Tejidos Vegetales. Alcalá de Henares (Madrid), Spain, 25-27 June 2007.
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