Micropropagation and in vitro grafting techniques to assist the selection of a pistachio rootstock from a population of terebinth (*Pistacia terebinthus* L.) collected in the SE of Spain

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Background	Pistachio culture is becoming highly interesting in Spain, where traditional crops as grape and olive have reduced their profitability. However, its expansion is hampered both by the difficulty of grafting varieties of interest on selected rootstocks, and by the lack of a selected clonal rootstock.
What	Collecting and selecting well adapted autochthonous clones of <i>P. terebinthus</i> in the wild. Applying in vitro culture techniques for the clonal propagation of <i>Pistacia</i> species as well as for studying factors affecting grafting.
How	Improving the germination protocol of <i>Pistacia</i> seeds. Optimizing the culture medium for both, scion and variety. Setting up a suitable grafting technique in vitro as a model to study grafting evolution.

Germination. Collected mature seeds from *P. terebinthus* trees were treated with Ethrel, Nufarm (2%), for 24 h at 4-5°C. Seeds were sowed in forestall cells containing a mixture of peat: coconut fiber: perlite. Germination data were recorded every other week and length and number of nodes at the 8th week.

Establishment. *P. terebinthus* shoots were taken from seedlings. Simultaneously, one-node explants of *P. vera* plants grown in the field were taken in spring and disinfected with 0.05% HgCl₂.

Multiplication. Three different media (DKW, WPM, MS) were tested. Shoots were subcultured every three weeks and culture media were compared attending to: apical necrosis (%), average shoot length (mm), number of internodes per shoot and multiplication rate (number of shoots per shoot).

Rooting. Shoots of *P. terebinthus* were rooted on two different media. Rooting percentage in WPM (78 shoots) or MS (24 shoots) was recorded after 4-8 weeks.

In vitro grafting. In vitro *P. vera* shoot tips (1-2 cm length) were grafted in vitro on rooted or unrooted shoots of *P. terebinthus* (4 cm length) with their apexes removed. Scions with the base cut in v-shape were fitted onto vertical slits made on the decapitated rootstocks. Homografts of *P. terebinthus* were also made as controls. Histological studies were undertaken after 4-8 weeks following grafting. Cell proliferation and differentiation at the grafting interface and vascular connections between rootstock and scion were recorded following 0.01% acridine orange stain (Pearse, 1968).

Germination of *P. terebinthus* seeds

Selected *P. terebinthus* trees (PT1, 2, 6, 8, 9, 10) showed different degree of germination capability and vigour.

Ethrel significantly improved germination rate as well as seedling growth, except in seeds from PT10, where the effect was adverse.

Micropropagation

Survival of *P. vera* initial nodal explants was 40%. Germination of *P. terebinthus* ranged from 27% to 80 % after 4 to 6 weeks.

DKW culture medium was better in reducing apical necrosis than MS or WPM, while obtaining good multiplication rates. Rooting was achieved in modified WPM medium in 82% of the shoots but decreased to 42% in modified MS medium. Plants were successfully transferred to the greenhouse.

	n	Length (mm)	Number of internodes	Multiplication rate	Apical necrosis(%)
<i>P. vera</i>					
DKW	111	23.68 ± 1.00a	6.3 ± 0.20a	2.5 ± 0.11b	25.22c
MS	112	17.46 ± 0.61c	4.9 ± 0.15b	2.1 ± 0.09c	47.32b
WP	112	20.64 ± 0.75b	5.1 ± 0.16b	3.5 ± 0.17a	61.61a
		***	***	***	***
<i>P. terebinthus</i>					
DKW	30	30.9 ± 3.25a	6.1 ± 0.36a	1.8 ± 0.13	10.0b
MS	30	19.8 ± 1.45b	4.9 ± 0.28b	1.8 ± 0.19	36.7a
WP	30	20.5 ± 1.48b	4.1 ± 0.28b	1.9 ± 0.15	26.7ab
		***	***	n.s.	*

Mean values (± s.e.m.) of shoot length, number of internodes, multiplication rate and the percentage of apical necrosis (*, ***, n.s.: P<0.05, P<0.001, not significant, respectively; n: sample size)

In vitro grafting

Most of the grafts showed cell proliferation and cell differentiation at the graft interface.

Despite the fact that the technique must be improved to reduce the presence of necrotic cells that are also present in *P. terebinthus* homografts, in vitro grafting studies opens a way to monitor graft evolution.

	n	Cell Proliferation	Cell Differentiation	Partial Contact	Cell Necrosis
<i>P. vera</i> / <i>P. terebinthus</i>	31	90 %	67%	80%	100%
<i>P. terebinthus</i> / <i>P. terebinthus</i>	5	100%	100%	80%	60%

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