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In vitro propagation of *Helianthemum almeriense* Pau (Cistaceae)

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Summary — Shoot tips and nodal segments were excised from seedlings grown *in vitro*. Outgrowth of axillary buds was achieved on Murashige and Skoog (MS) medium supplemented with 0.46 μM kinetin without auxins. Cultures established from shoot tips and nodal segments had the same multiplication rate. Spontaneous rooting was observed at a low cytokinin concentration until the 6th subculture. After that, spontaneous rooting was negligible. The rooting percentage increased to 92% *via* macronutrient dilution. The rooted shoots were grown in a peat–sand–vermiculite mixture.

***in vitro* propagation / *Helianthemum* (Cistaceae)**

Résumé — Propagation *in vitro* de *Helianthemum almeriense* Pau (Cistaceae). *Des extrémités de tiges et des segments nodaux ont été excisés de semis cultivés* in vitro. *La croissance de bourgeons axillaires a été obtenue sur le milieu de Murashige et Skoog (MS) avec 0.46 mmol.l^{-1} de kinétine sans auxine. Les cultures établies à partir d'extrémités de tiges ou de segments nodaux ont eu le même taux de multiplication. L'enracinement spontané a été observé à faible concentration de cytokinine jusqu'à la 6e subculture. Ultérieurement, l'enracinement spontané est devenu négligeable. Le pourcentage d'enracinement a augmenté jusqu'à 92% en diluant les macroéléments. Les tiges enracinées ont été cultivées sur un mélange de tourbe, sable, vermiculite.*

propagation* in vitro / Helianthemum *(Cistaceae)

INTRODUCTION

Helianthemum almeriense Pau (Cistaceae) is one of the most abundant autochtonous shrubs of the semi-arid areas in Spain. This crop is of great interest for reafforestation. It establishes endomycorrhizae with Ascomycetes such as *Terfezia* and *Picoa* (desert truffles) (Honrubia *et al*, 1992) which are of marked gastronomic and economic importance in the area. In this paper, we have established a protocol for *in vitro* propagation of this crop.

MATERIALS AND METHODS

Mature *H almeriense* seeds were surface-sterilized by pre-treatment for 10 s in a 70% ethanol solution, followed by treatment for 20–30 min in 10% commercial bleach ("Domestos") solution, followed by rinsing in 80% ethanol for 10 s and 3 times 5-min rinses in sterile distilled water.

Basal medium consisted of Murashige and Skoog (1962) (MS) salts supplemented with myo-inositol (100 mg.l^{-1}) thiamine–HCl (0.1 mg.l^{-1}), nicotinic acid (0.5 mg.l^{-1}), pyridoxine–HCl (0.5 mg.l^{-1}) and 3% sucrose. pH was adjusted to 5.8 before autoclaving. The medium was solidified with 7 g.l^{-1} Panreac agar and autoclaved for 20 min at 121 °C and 103 kPa. Naphthalene acetic acid (NAA) and 6-benzylaminopurine (BAP) and kinetin were added as indicated.

Seeds were sown on hormone-free MS medium and germinated under a 16-h photoperiod at 25 °C. Shoot tips and nodal segments were excised from the seedlings and transferred to multiplication medium. Thirty cultures per treatment were grown at 25 ± 2 °C and 40 μmol.m^{-2}.s^{-2} Growlux fluorescent light and a 16-h photoperiod. Experiments were repeated at least 3 times.

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Following shoot multiplication and elongation, 15–25 mm shoots were transferred to root induction medium consisting of MS medium salts at several strengths (1x, 1/2x, 1/4x) with or without activated charcoal (2 $g.l^{-1}$). After 6 wk, plantlets with well developed roots were potted in a peat–sand–vermiculite mixture (6:2:2, v/v). The plantlets were adapted to growth chamber conditions (25 ± 2°C) with a 16-h photoperiod. They were gradually exposed to reduced relative humidity by progressively removing a plastic cover during a period of 2–3 wk. Once acclimatization was accomplished, the plants were transferred to the greenhouse.

RESULTS

After 2 wk in MS medium without growth regulators, most seeds (75%) had germinated. Production of axillary shoots began after 2 wk when kinetin or BAP had been added. Cultures established from shoot tips (1 cm) and nodal segments (0.5–1 cm) had the same multiplication rate. The maximum number of microshoots per explant was obtained with 0.46 or 0.93 μM kinetin (table I, fig 1). Progressively higher concentrations of BAP alone (1.78, 2.66, 3.55 μM) or in combination with 0.27 μM NAA (0.44,

Table I. The influence of BA, kinetin and NAA on number of microshoots produced from shoot tips and nodal segments of *H almeriense* cultured for 12 wk *in vitro* (data based on 30 cultures/treatment).

MS medium + growth regulator (μM)			*No of microshoots/ explant*
BAP	*Kinetin*	*NAA*	
0.22	0.0	0.0	2.48^{abc}
0.44	0.0	0.0	2.73^{abc}
0.88	0.0	0.0	2.29^{abc}
1.33	0.0	0.0	2.79^{abc}
1.78	0.0	0.0	0.99^{a}
2.66	0.0	0.0	1.31^{ab}
3.55	0.0	0.0	1.66^{ab}
0.22	0.0	0.27	2.91^{bc}
0.44	0.0	0.27	2.33^{abc}
0.88	0.0	0.27	2.32^{abc}
1.33	0.0	0.27	1.86^{ab}
0.0	0.46	0.0	7.72^{d}
0.0	0.93	0.0	6.12^{d}
0.0	1.39	0.0	4.08^{c}

Data in a column followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Duncan's test.

Fig 1. Shoots of *Helianthemum almeriense* proliferated on basal medium with 0.46 μM of kinetin.

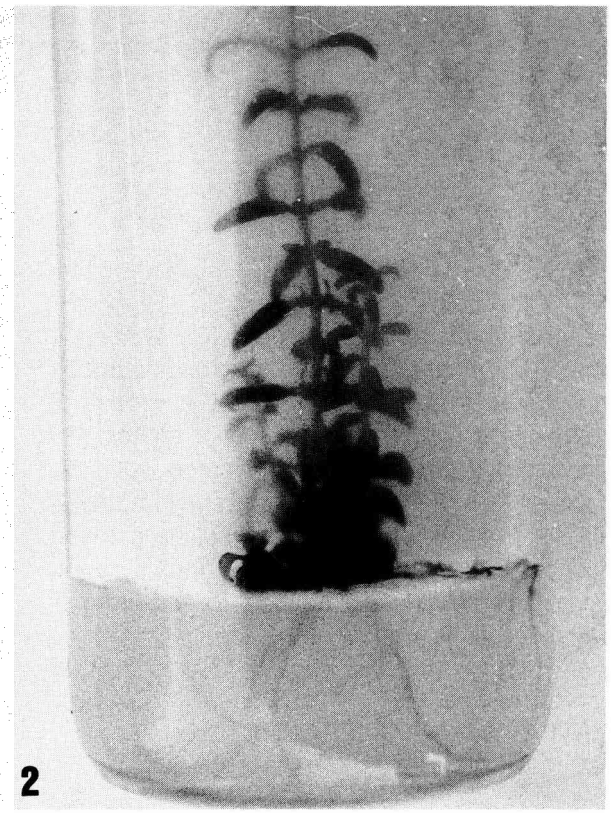

Fig 2. Formation of roots at the base of a *Helianthemum almeriense* plantlet.

0.88, 1.33 μM) resulted in a decrease in the number of shoots. Callus was induced at 3.55 μM BAP and 1.33 μM BAP with 0.27 μM NAA. A slight decrease in the propagation rate was observed after the 4th subculture (5.6 microshoots per explant).

Spontaneous rooting was observed at a low cytokinin level until the 6th subculture (fig 2). After that rooting was negligible. To avoid necrosis during rooting, it was necessary to select shoots between 15–25 mm long. Rooting percentage was enhanced to 92% by macronutrient dilution (fig 3). Addition of charcoal reduced rooting. Approximately 95% rooted plants transferred to soil survived. All these plants were first grown in the greenhouse and afterwards in the field. They showed normal development and flowering.

DISCUSSION AND CONCLUSION

The present report shows that *in vitro* methods are a feasible means to propagate and obtain true-to-type plants of *Helianthemum almeriense.* Of the 2 cytokinins tested, kinetin was more efficient than BAP. When used at a low concentration (0.46 μM), it allowed good growth of the shoot which is particularly critical when shoot multiplication through axillary branching is carried out by taking nodal segments at each subculture. In this case the rate of shoot multiplication is directly related to the elongation of shoots and the number of nodal cuttings available at the end of each passage (Bhojwani and Razdan, 1983). This also allowed exclusion of an additional *in vitro* step of shoot elongation on a medium with low concentration of cytokinin before rooting.

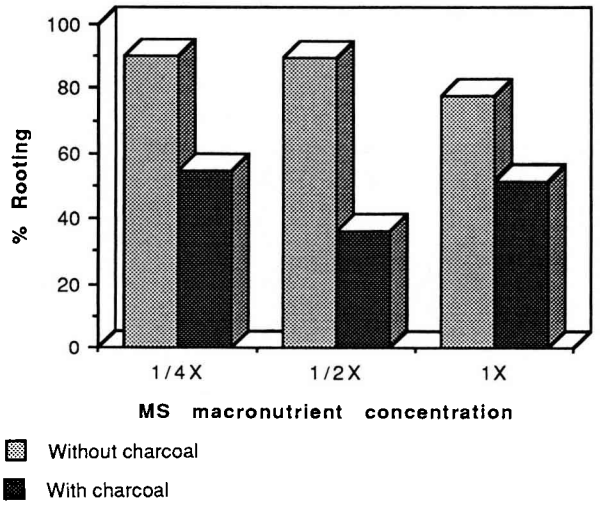

Fig 3. Influence of concentration of macronutrients and addition of charcoal on *in vitro* rooting in medium without growth regulators after 4 wk (48 explants per treatment).

Quoirin *et al* (1977) showed that dilution of macronutrients improved the rooting of several *Prunus* species. M'Kada *et al* (1990) observed the same in *Cistus* x *purpureus.* We also observed an increase in rooting after dilution (fig 3). Contrary to Cheema and Sharma (1983) and M'Kada *et al* (1990), charcoal decreased root production. The effect of charcoal may be related to the adsortion of endogenous auxins which promote rooting.

The propagation of *Helianthemum almeriense* by tips and nodal microcuttings is a very efficient method. However, an economic study would have to be performed to determine whether this process is more advantageous than traditional seed propagation.

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