Micropropagation of Six Rockrose (*Cistus*) Species

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Abstract. Micropropagation methods for six rockrose species (*Cistus albidus* L., *C. clusii* Dunal, *C. ladanifer* L., *C. laurifolius* L., *C. psilosepalus* L., and *C. salvifolius* L.) were established. Cultures, initiated from nodal segments of seedlings, were grown on MS medium, alone, or supplemented with 0.88 μM BAP or 0.93 μM Kin. Multiple shoot formation was obtained after the first subculture (30 days) from which new nodal segments were taken and grown on the same culture medium to maintain proliferation. Shoots obtained at the third subculture were rooted alone or supplemented with different concentrations of IBA. The plantlets of the six species, thereby obtained, were successfully acclimatized to ex vitro conditions. Chemical names used: 6-benzylaminopurine (BAP), indole-3-butyric acid (IBA), 6-furfurylaminopurine (Kin).

The Cistaceae family is comprised of eight genera and ≈ 165 species distributed throughout the temperate regions in the northern hemisphere and South America. It is found in the Mediterranean Basin, where it forms an important part of the shrubs, especially in noncalcareous soils where it is widely distributed.

Several *Cistus* spp. are used as ornamentals for their flowers, produced in abundance and notable for their size and singular beauty. In the United States, *Cistus* spp. have a great potential in the west and southwest due to their drought tolerance. Rockroses are regarded as melliferous flora, the honey obtained from them being of great economic interest. A gum (ladanum) also is obtained from some *Cistus* spp., especially *C. ladanifer*. Ladanum, traditionally used in medicinal plasters, is used extensively in perfumery (Brickell, 1989; López, 1982; Polunin and Huxley, 1972). A novel use for *Cistus* spp. involves inoculating them with the mycorrhizae of *Tuber nigrum* Bull (a black truffle) and planting them in a primary stage of truffle forest repopulation (Pacioni, 1987).

Cistus spp. multiply naturally by seed, although germination is low and erratic due to hard-seededness (Thanos and Georgiou, 1988). The diverse hybrids and cultivars used in gardening are multiplied exclusively by softwood cuttings and layering (Brickell, 1989). However, vegetative propagation is difficult in most wild *Cistus* species (Sigüero, 1991). Micropropagation of six *Cistus* species was attempted as an alternative for the propagation of wild *Cistus* germplasm, which may prove useful in initial selection stages of a breeding program. Our objective was to develop a simple micropropagation system ap-

plicable to a wide range of genotypes in each species. To our knowledge, there are few studies on the micropropagation of Cistaceae (M'Kada et al., 1991; Morte and Honrubia, 1992).

Materials and Methods

To overcome hard-seededness, mature seeds of *C. albidus*, *C. clusii*, *C. ladanifer*, *C. laurifolius*, *C. psilosepalus*, and *C. salvifolius* were subjected to a dry-heat pretreatment at 100C for 10 to 30 min, depending on the species (Corral et al., 1989; Thanos and Georgiou, 1988; Trabaud and Oustric, 1988, 1989). Seeds then were surface-sterilized by immersion in a solution of 1.5% NaOCl for 30 min, followed by three rinses of 5 min each with sterile distilled water.

Basal medium consisted of MS medium (Murashige and Skoog, 1962), 3% sucrose, and 0.7% Difco Bacto agar. The pH was adjusted to 5.8 before autoclaving (121C for 20 min). Seeds were sown on MS basal medium without growth regulators. Thirty days after sowing, nodal segments (0.5 to 1 cm) were excised from seedlings and placed on three media: MS basal medium, MS + 0.93 μM Kin, or MS + 0.88 μM BAP. Every 4 weeks, nodal segments (0.5 to 1 cm) were subcultured to the same media. Microshoots from the third subculture were transplanted to MS basal medium or MS supplemented with IBA (0.49, 0.98, 1.97, 2.95, 3.94, and 4.90 μM) for rooting. In all micropropagation steps, 7 $\times$ 8-cm glass culture vessels, containing 30 ml of culture medium, were used.

Rooted plantlets were washed to remove agar, and roots were sprayed with a 0.3-g-liter⁻¹ carbendazim solution [0.6 g Bavistin/liter (BASF Española, Barcelona, Spain)]. Then the plantlets were transferred to 0.5-liter pots containing a 5 sterilized peat : 2 vermiculite mixture (v/v), covered with inverted jars to avoid excessive water loss, and maintained under greenhouse conditions. The plants were irrigated every 2 days with water. Jars were removed 6 weeks after transfer to pots, and 1 week later, the plants were transplanted to soil in the field.

All cultures were grown at 25 $\pm$ 1C under a 16-h photoperiod with a light intensity of 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by cool-white fluorescent lamps. At least 20 explants were used for each treatment and each experiment was conducted three times. Analysis of variance and Duncan's multiple range test ($P < 0.05$) were performed on the number of shoots per explant. Polynomial regressions were applied to the percentage of rooted microcuttings and the number of roots per microcutting.

Results and Discussion

After 30 days in culture, shoot proliferation was evident for all six *Cistus* species in the three media. *Cistus clusii* had the lowest proliferation percentages (73% to 82%), whereas the values were between 80% and 97% for the other five species.

Growth regulator type had a significant effect on the number of shoots per explant after three subcultures, and results varied with the species (Table 1). In *C. albidus*, *C. laurifolius*, and *C. salvifolius*, the highest number of shoots per explant were obtained when the MS medium was supplemented with 0.88 μM BAP. The response to MS and MS + 0.88 μM BAP was similar for *C. clusii*, *C. ladanifer*, and *C. psilosepalus*. In all species, except *C. psilosepalus*, the number of shoots per explant in MS + 0.93 μM Kin was always significantly lower ($P \leq 0.05$) than for MS + 0.88 μM BAP. This result differed from those of Morte and Honrubia (1992) with nodal segments of seedlings of *Helianthemum almeriense* Pau, where adding 0.93 μM Kin to MS medium produced a significantly higher number of shoots per explant than adding 0.88 μM BAP.

In most cases, shoots were significantly shorter with Kin (0.93 μM) in the culture medium than with BAP (0.88 μM) or without growth regulators (data not shown). There also were no significant differences attributed to the subculture in this study.

Adventitious roots formed in all six species in all the treatments in MS medium without auxins, with the exception of *C. salvifolius*.

Table 1. Effects of BAP or Kin on number of shoots per explant in six *Cistus* species after 4 weeks in culture.^z

Growth regulator (μM)	No. shoots/explant					
	<i>C. albidus</i>	<i>C. clusii</i>	<i>C. ladanifer</i>	<i>C. laurifolius</i>	<i>C. psilosepalus</i>	<i>C. salvifolius</i>
Control	1.8 b	2.5 a	2.8 a	1.1 b	3.0 a	1.7 c
0.93 Kin	2.0 b	1.6 b	2.0 b	1.2 b	3.1 a	2.6 b
0.88 BAP	2.8 a	2.3 a	2.7 a	2.5 a	3.2 a	4.8 a

^zMean separation in columns by Duncan's multiple range test at $P \leq 0.05$.

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Table 2. Effects of IBA on the percentage of rooted microcuttings and the number of roots per microcutting for six *Cistus* species after 4 weeks in culture.

IBA (μM)	% Rooted microcuttings ^z						No. roots/microcutting ^z					
	<i>C. alb</i>	<i>C. clu</i>	<i>C. lad</i>	<i>C. lau</i>	<i>C. psi</i>	<i>C. sal</i>	<i>C. alb</i>	<i>C. clu</i>	<i>C. lad</i>	<i>C. lau</i>	<i>C. psi</i>	<i>C. sal</i>
0.00	72	54	75	40	95	2	2.8	2.5	5.2	4.1	7.4	---
0.49	70	52	84	51	75	32	2.7	3.5	5.5	3.9	6.5	2.7
0.98	80	55	82	74	86	49	2.3	2.3	5.1	3.1	7.4	4.1
1.97	84	46	92	67	82	48	3.4	2.1	5.7	2.8	6.6	3.8
2.95	81	71	88	68	90	41	3.6	2.7	5.1	4.1	7.2	2.5
3.94	67	71	87	67	99	41	3.2	2.5	5.3	5.2	8.4	2.5
4.90	78	77	78	50	90	42	3.6	2.3	4.7	3.1	8.3	4.4
Regression												
Linear	NS	**	NS	NS	*	*	***	NS	NS	NS	**	NS
Quadratic	NS	**	*	NS	*	**	***	NS	NS	NS	***	NS

^z*C. alb* = *C. albidus*; *C. clu* = *C. clusii*; *C. lad* = *C. ladanifer*; *C. lau* = *C. laurifolius*; *C. psi* = *C. psilosepalus*; *C. sal* = *C. salvifolius*.NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

(Table 2). Percentage of rooted microcuttings was significantly affected by IBA treatments in *C. clusii*, *C. ladanifer*, *C. psilosepalus*, and *C. salvifolius*, whereas the number of roots per explant was affected significantly only by IBA treatments in *C. albidus* and *C. psilosepalus*. Thus, the response to the range of IBA concentrations studied was particular for each species. Genotype also seemed to influence the overall rooting capacity. *Cistus salvifolius* was more difficult to root, whereas *C. psilosepalus* and *C. ladanifer* showed high rooting capacity.

In *H. almeriense*, it was necessary to select shoots longer than 15 mm for rooting to avoid necrosis and death (Morte and Honrubia, 1992). Shoots also increased considerably in length in the rooting media. In our case, this effect was absent in all six species studied.

Survival after acclimatization was assessed 10 weeks after the transfer of plantlets to ex vitro conditions. The highest survival rates were obtained with *C. psilosepalus* (77%), followed by *C. salvifolius* (65%), *C. ladanifer* (62%), *C. albidus* (57%), and *C. clusii* (44%), the lowest by *C. laurifolius* (36%).

A simple procedure has been developed for

micropropagation in six rockrose species of economic interest for use in the initial selection stages of a breeding program. All species responded to MS medium containing 0.88 μM BAP for multiple shoot production. Easy-to-root species (*C. albidus*, *C. ladanifer*, and *C. psilosepalus*) rooted well on a medium without IBA. *Cistus clusii*, *C. laurifolius*, and *C. salvifolius* rooted best with IBA (0.49 to 4.9 μM) included in the medium. IBA at 4.9 μM was an effective treatment for all species. Additional research is warranted to develop micropropagation methods starting from mature explants, which would be useful for the propagation of advanced cultivars obtained from this germplasm.

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