Rooting experiments with *Euphorbia lagascae* cuttings

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Introduction

*Euphorbia lagascae* Spreng. is an spurge wildly present at Southeastern Spain, it produces 50% of seed oil with 60% of cis 12,13-epoxy oleic or vernolic acid (Kleiman et al. 1964) which has several applications in the plastic and electronic industries. This species has been evaluated as a potential new oilseed crop (Pascual-Villalobos et al. 1994, Turley et al. 2000).

In the 1990s mutation breeding work was undertaken and the screening of indehiscent genotypes was carried out in M₁ generation of chemically treated seeds of *E. lagascae*. This species is a seed propagated annual plant but for mass selection, it could have been helpful to have an easy vegetative propagation system to increase the number of plants with the selected character.

Nowadays a breeding objective in *Euphorbia lagascae* would be the reduction of skin irritant compounds in the latex (Turley et al. 2000) and a similar procedure, screening M₂ populations would apply. Again a vegetative propagation method could speed up the selection process.

Successful in vitro vegetative propagation has been reported for perennial *Euphorbia* species (Langehe et al. 1974, Lee et al. 1982, Jacobek et al. 1986, Zhang et al. 1987, Nielsen et al. 2003).

Abstract

To study the potential of *Euphorbia lagascae* for vegetative propagation, in vivo rooting assays were carried out using cuttings from apical and axillary shoots. Indolbutyric acid basal immersion (50mg/l) during 2 min of axillary cuttings was the most effective treatment in terms of survival (100%) and shoot lenghth (20,5 cm) in comparison with the untreated control (50% survival and 8 cm long shoots).

Key words: Auxin, Euphorbiaceae, Spurge, Vegetative propagation
In this study rooting experiments were carried out for the first time with *E. lagascae* cuttings to test the response of this species to vegetative propagation in vivo.

Among auxins, IBA and NAA are the most used and cited in bibliography because of their low price and their effectiveness on plant survival, growth and rooting (Douglas 1984, Gray & Benton 1991, Pardo 1994).

**Results and Discussion**

Survival was only successful (80% - 100%) with IBA basal immersion during 2 min. Two treatments, NAA 1 min and IBA 15 seg, to axillary shoots produced 0% survival. Significant differences in shoot length among treatments were only observed after two months (Table 1). Apical shoots treated with NAA for 1 min. produced the greatest growth.

Hormonal treatment increased the number of roots after one and two months compared to control. However differences in root length were only evident after two months. No effect of hormone, time of immersion or cutting type on the number of roots was observed after one month. After two months, the following results were observed: a) apical shoots produced higher number of roots (average 13) than axillary shoots (average 8), b) IBA applications were more effective than NAA (average number of roots of 15.6 against 10.3), c) there was an interaction between hormone and time since longer immersion times were effective with NAA but not with IBA-in terms of number of roots formed, d) hormonal treatments did not differ in root length (average 10.8) among them but were significantly different from control treatments (5.8) and e) rooting scale records indicated that IBA applications produced a better rooting system than NAA treatments, and both than the control.

IBA has been reported as a stronger auxin than NAA on promoting rooting (Lê 1985, Németh 1986, Gray & Benton 1991) despite of the concentration used (0.21 mM) in this experiment was lightly lower than that used for NAA (0.27 mM).

Probably, the apical dominance of the apical main shoot could affect rooting due to the different auxin or cytokinin endogenous contents respect to the axillary shoots (Norton 1986, Diaz-Sala 1989, Pandeliev 1990, Nicolau 1991). *Euphorbia lagascae* apical shoots could have an endogenous content of auxins different to axillary shoots and this can influence the result (Wellander 1988, Sudarsono & Goldy 1991, Wang 1991, Marks & Myers 1992).

The results demonstrate that *E. lagascae* vegetative propagation is feasible, however, new experiments including other factors such as hormone concentration, environmental conditions and different seasons must be tried together with a following up of field survival after propagation. In this study, when cuttings were transplanted to the open field, it was

**Material and methods**

Cuttings of *E. lagascae* (2-3cm long) with 4-10 leaves were obtained from plants (45 days old) growing in benches in the open field. Two types of cuttings, apical (Ap) and axillary (Ax) shoots (cotyledonary sprouts) were placed in glass flasks with distilled water and carried to the laboratory. Plant material was surface sterilized with 1.5% sodium hypochlorite for 10 min and rinsed three times (10 min each) with sterilized distilled water.

A bifactorial experiment of 10 treatments was set up including cutting type and hormonal treatment in a completely randomized design with 10 replications. Hormonal treatments consisted of basal immersion of cuttings in hormone solutions of indolbutyric acid (IBA) or naphthaleneacetic acid (NAA) at 50 mg-liter⁻¹ in distilled water (pH 6.5). Two immersion times were applied in each case, 15 seg or 2 min for IBA and 15 seg or 1 min for NAA solutions. The control treatments were hormone free.

Once treated, each cutting was placed in a pot (10 x 6 cm) filled up with sterilized potting (peat-perlite, 1:1, v/v) medium, and covered with a transparent plastic bag and maintained in a chamber at 24ºC, 85%RH and 16:8 photoperiod (light:dark) between 3,000-3,500 luxes. The plastic bags were progressively opened after the third week and irrigation was applied every two days up to the sixth week when they were removed. After seven weeks, plantlets were transferred to another chamber at lower temperature (20ºC) and higher light intensity (8000 luxes) with 85% RH. When plants were two months old they were placed in a shaded bench (70% light exclusion) where they stayed until plants (three months and a week old) were transplanted to the open field.

Survival (%), root and shoot length (cm) and number of leaves and roots were recorded after 1 and 2 months. Also a rooting scale (from 1 to 3) was given at the end of the experiment to show the root development. After establishment in the open field, vegetative and reproductive development of plants was observed.

Data were analyzed by Analysis of Variance (ANOVA) and Duncan’s multiple range test for mean separation (P<0.01).
### Table 1. Effect of hormonal treatment in survival, growth and rooting of *E. lagascae* cuttings.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>After 1 month</th>
<th></th>
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<th>After 2 months</th>
<th></th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Roots</td>
<td></td>
<td>Shoot</td>
<td>Roots</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Survival (%)</td>
<td>Length (cm)</td>
<td>Leaves (nº)</td>
<td>Number</td>
<td>Length (cm)</td>
<td>Leaves (nº)</td>
<td>Number</td>
</tr>
<tr>
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<td>2a</td>
<td>3a</td>
<td>1a</td>
<td>2,5a</td>
<td>50</td>
<td>8a</td>
</tr>
<tr>
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<td>3a</td>
<td>3a</td>
<td>0a</td>
<td>-</td>
<td>40</td>
<td>9,8a</td>
</tr>
<tr>
<td>NAA 15seg Ax</td>
<td>50</td>
<td>10a</td>
<td>5a</td>
<td>3b</td>
<td>2a</td>
<td>50</td>
<td>19,5b</td>
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<tr>
<td>NAA 15seg Ap</td>
<td>60</td>
<td>7,5a</td>
<td>7a</td>
<td>2,3b</td>
<td>3,2a</td>
<td>40</td>
<td>11,8a</td>
</tr>
<tr>
<td>NAA 1 min Ax</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0-</td>
<td>-</td>
</tr>
<tr>
<td>NAA 1 min Ap</td>
<td>40</td>
<td>4,5a</td>
<td>6a</td>
<td>3,5b</td>
<td>3a</td>
<td>40</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
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<td>7a</td>
<td>3b</td>
<td>2a</td>
<td>20</td>
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<td>4a</td>
<td>3a</td>
<td>3b</td>
<td>2a</td>
<td>100</td>
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<tr>
<td>IBA 2 min Ap</td>
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<td>2,5a</td>
<td>5a</td>
<td>4b</td>
<td>2a</td>
<td>80</td>
<td>14,7a</td>
</tr>
</tbody>
</table>

*Cutting type: Ax = axillary shoot and Ap = apical shoot*

*Duncan’s multiple range tests by columns (P<0.01)*

*Rooting scale: 1 = good; 2 = medium; 3 = poor*
observed that untreated plants and plants from cuttings of axillary buds flowered irregularly. In other cases, the plants flowered and produced fruits normally. This question must be studied in future research.

Other possibility, as Zhang & Stoltz (1989) and Erstad & Gislerod (1994) reported, is rooting after in vitro multiplication which can be more successful than conventional methods.

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References


