Anales de Biología 26: 101-104, 2004

Rooting experiments with *Euphorbia lagascae* cuttings

Ascensión Ibáñez-Torres
D.G. de Regadíos y Desarrollo Rural, Consejería de Agricultura y Agua, Plaza Juan XXIII, s/n. 30008 Murcia, Spain.

Correspondencia
A. Ibáñez
E-mail: Ascension.ibanez@carm.es
Tlfno: 968 36 83 80; 699 65 36 16.
Fax : 968 36 28 61.
Received: 10 June 2004
Accepted: 22 September 2004

Resumen

Experimentos de enraizamiento con estaquillas en Euphorbia lagascae*.*

Se realizó un ensayo de enraizamiento *in vivo* con estaquillas axilares y terminales de *Euphorbia lagascae*, utilizando distintos reguladores de crecimiento (NAA, IBA, a 50 mg/l) y distintos tiempos de actuación de los mismos. A la vista de los resultados se observó que el mejor tratamiento fue IBA aplicado durante 2 minutos, y el mejor tipo de explanto fueron esquejes axilares, obteniendo una supervivencia del 100% y longitud del brote de 20,5 cm, en comparación con un 50% de supervivencia y brotes de 8 cm en el testigo, a los dos meses del tratamiento.

Palabras clave: Auxina, Euphorbiaceae, Euforbia, Propagación vegetativa

Abstract

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

Key words: Auxin, Euphorbiaceae, Spurge, Vegetative propagation

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 M_2 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 (Langhe et al. 1974, Lee et al. 1982, Jacobek et al. 1986, Zhang et al. 1987, Nielsen et al. 2003).

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).

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 ($P<0.01$).

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 moths 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 an 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/and 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

Mean values [y]											
	After 1 month					After 2 months					
	Shoot			Roots		Shoot			Roots		
Treatments	**Survival (%)**	**Lenght (cm)**	**Leaves (nº)**	**Number**	**Length (cm)**	**Survival (%)**	**Lenght (cm)**	**Leaves (nº)**	**Number**	**Length (cm)**	**Rooting scale [x]**
Control Ax	50	2a	3a	1a	2,5a	50	8a	19a	4a	5a	3a
Control Ap	40	3a	3a	0a	-	40	9,8a	22a	7,5b	6,5a	3a
NAA 15seg Ax	50	10a	5a	3b	2a	50	19,5b	34a	4a	9,8b	2b
NAA 15seg Ap	60	7,5a	7a	2,3b	3,2a	40	11,8a	30a	10,5b	10b	2,5b
NAA 1 min Ax	0	-	-	-	-	-	-	-	-	-	-
NAA 1 min Ap	40	4,5a	6a	3,5b	3a	40	34,8c	33a	16,5c	7,8b	2b
IBA 15seg Ax	0	-	-	-	-	-	-	-	-	-	-
IBA 15seg Ap	20	3a	7a	3b	2a	20	14,5a	36a	22c	13,7b	1,5c
IBA 2 min Ax	100	4a	3a	3b	2a	100	20,5b	25a	16c	13b	1,5c
IBA 2 min Ap	80	2,5a	5a	4b	2a	80	14,7a	33,5a	8.8b	10,2b	1c

[z] Cutting type: Ax = axillary shoot and Ap = apical shoot
[y] Duncan's multiple range tests by columns (P<0.01)
[x] Rooting scale: 1 = good; 2 = medium; 3 = poor

Table 1. Effect of hormonal treatment in survival, growth and rooting of *E. lagascae* cuttings.[z]

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.

Acknowledgments

The author thanks María Jesús Pascual-Villalobos for her suggestions in this experiment and Asunción Morte for her useful comments to the manuscript. This work was part of the SONCA/ ÉCLAIR/CEE project, and was carried out at Instituto Murciano de Investigación y Desarrollo Agroalimentario.

References

Díaz Sala C.1989. Posible efecto inhibidor de medios de proliferación sobre el posterior enraizamiento «in vitro» de peral (cv.Guyot). FYTON 50 (1/2): 73-80.

Douglas GC. 1984. Propagation of eight cultivars of Rhododendron «in vitro» using agar solidified and liquid media and direct rooting of shoot «in vivo». Scientia Horticulturae 24: 337-347.

Erstad JLF & Gislerod HR.1994.Water-uptake of cuttigs and stem pieces as affected by different anaerobic conditions in the rooting medium. Scientia Horticulturae-Amsterdam 58 (1-2): 151-160.

Gray DJ & Benton CM.1991. «In vitro» micropropagation and plant establishement of Muscadine grape cultivars, Vitis rotundifolia. Plant Cell Tissue and Organ Culture 27: 7-14.

Jakobek J.L, Backhaus RA & Herman K. 1986. Micropropagation of candelilla. Euphorbia antisyphillitica Zucc. Plant Cell Tissue and Organe Culture (NLD) 7(2): 145-148.

Kleiman R, Smith CR & Yates S.G. 1964.Search for new industrial oils. XII. Fifty eight Euphorbiaceae oils, including one rich in vernolic acid. Journal of the American Oil Cheemists Society 42: 169-171.

Langhe ED, Debergh P & Van Rijk R. 1974. In vitro culture as a method for vegetative propagation of Euphorbia pulcherrima. Zeitschrift fur Pflanzenphysiologie 71:271-274.

Lê CL. 1985. La micropropagation. Revue Suisse Viticultute Arboriculture y Horticulture 17 (6): 347-349.

Lee CW, Jeckes J & Thomas JC.1982.Culture Propagation of Euphorbia lathyris. HortScience 17(3): 533.

Marks TR & Myers PE.1992. Effect of explant location upon early culture development «in vitro». Journal of Horticultural Science 67 (5): 583-591.

Németh G. 1986. Induction of rooting. In Biotechnology in Agriculture and Forestry, vol,1. Trees (Bajaj YPS, ed). Berlin Heidelberg: Springer-Verlag, pp 49-64.

Nicolau NA. 1991. Le choix des explants et l´accélération de la croissance des tiges de Vigne en culture «in vitro». Progreso Agricicola et Viticola 108: 235-238.

Norton ME.1986. Explant origin as a determinant of «in vitro» shoot proliferation in Prunus and Spiraea. Journal of Horticultural Science 61: 43-48.

Nielsen MD, Farestveit B & Andersen AS. 2003.Adventitious shoot development from decapitated plants of peri-clinal chimeric poisettia plants (Euphorbia pulcherrima Willd ex Klotsch). European Journal of Horticultural Science 68 (4): 161-168.

Pandeliev S, Rusueva RM & Georgieva P. 1990. Degree of development of vine plants under «in vitro» conditions depending on the biology of the initial explant. Rastenievudni Nauki Sofia (Summary) 27 (7): 79-83.

Pardo A.1994. Problemática del enraizamiento de especies difíciles. XXVI Jornadas de estudio: propagación vegetal el reto de las nuevas técnicas frente a los problemas actuales. Asociación Interprofesional para el Desarrollo Agrario. Zaragoza: AIDA, pp:199-216.

Pascual-Villalobos MJ, Röbbelen G & Correal E. 1994. Production and evaluation of indehiscent mutant genotypes in Euphorbia lagascae.Industrial Crops and Products 3: 129-143.

Sudarsono A & Goldy R. 1991. Growth regulator and axillary bud position effects on «in vitro» establishement of Vitis rotundifolia. Hortscience 26: 304-307.

Turley D, Froment M & Cook S. 2000. Development of Euphorbia lagascae as a new industrial oil crop. ADAS, woodthorne, wolverhampton uk, pp 38.

Wang Q.1991. Factors affecting rooting of microcuttings of the pear rootstock BP 10030. Scientia Horticulturae 45: 209-213.

Welander M.1988. Biochemical and anatomical studies of Birch (Betula pendula Roth) buds exposed to different climatic conditions in relation to growth «in vitro». In: Genetic manipulation of woody plants (Hanover JW & Keathley DE, eds.). Plenum Publishing Corporation, pp. 79-99.

Zhang B & Stoltz LP.1989. Acclimatization Systems for Euphorbia fulgens microcuttings. HortScience 24: 1025-1026.

Zhang B, Stoltz LP & Snyder JC.1987. In vitro propagation of Euphorbia fulgens. HortScience 22: 486-488.