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PLANT REGENERATION AND SHOOT PROLIFERATION FROM CULANTRO (*Eryngium foetidium* L.)

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ABSTRACT

A procedure for shoot regeneration from root, inflorescence-node, flower and leaf explants of culantro (*Eryngium foetidium* L.), is described. Root, inflorescence-node and flower explants were cultured on Murashige and Skoog medium (MS) alone or supplemented with 1, 2, 3, 4 or 5 mg/l benzyladenine (BA) with 0.5 mg/l naphthaleneacetic acid (NAA). Leaf explants were cultured on MS medium supplemented with 0.5, 3, 6 or 10 mg/l 2,4 dichlorophenoxyacetic acid (2,4-D), 3 mg/l 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid (Picloram), 3 mg/l NAA, 3 mg/l naphthoxyacetic acid (NOA) or 6 mg/l indoleacetic acid (IAA). Root, inflorescence-node and flower explants produced multiple shoots on BA-containing medium. Shoot number, shoot length and root number were influenced by BA and NAA concentrations. Callus was produced from leaf explants cultured on 2,4-D, picloram, NAA and NOA. Shoots were regenerated from callus on MS containing 0.5 mg/l BA. Shoots were rooted and transferred to soil and normal phenotypic plants were successfully established in soil.

Abbreviations: BA: benzyladenine, IAA: indoleacetic acid, MS: Murashige & Skoog's (1962) medium, NAA: naphthaleneacetic acid, NOA: naphthoxyacetic acid, Picloram: 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid, 2,4-D: 2,4 dichlorophenoxyacetic acid.

Key words: *coriander substitute, herb, medicinal plant , spices, tissue culture.*

1. INTRODUCTION

Culantro (*Eryngium foetidum* L.), a member of the family Umbellifera, is an aromatic herbal plant. It is used as a condiment and as a substitute for coriander in many countries in tropical Africa, Asia and Central America (Kuebel and Tucker, 1988). The essential oil of the aboveground parts was analyzed. Nineteen components were identified, of which the major ones were 2-dodecenal and 2-dodecenoic acid. The other constituents of this oil were also mainly aldehydes and acids (Leclercq *et al.*, 1992; Wong *et al.*, 1994; Pino *et al.*, 1997). Culantro is also used as medicinal plant since its leaves contain antiinflammatory and analgesic compounds (Saenz, *et al.*, 1997; Garcia, *et al.*, 1999).

Plant regeneration from leaves of culantro was described by Mohamed-Yasseen (1994) using thidiazuron and BA (Mohamed-Yasseen, 2001). Plant regeneration was also reported using leaf explants (Arockiasamy and Ignacimuthu, 1998; Ignacimuthu *et al.*, 1999). However, there is no available information about culantro plant regeneration from root, inflorescence-node or flower explants.

Culantro is propagated from seeds. Nevertheless, this method is a slow process and produces plants that are not identical. Tissue culture of culantro can be used for many purposes such as rapid plant propagation, cloning of elite plants, production of secondary products and improving plant quality through somaclonal variation or recombinant DNA. The purpose of this work is to establish an efficient system for plant regeneration through direct shoot formation, for clonal propagation. In addition, it investigates the effects of several auxins on callus formation. Callus is beneficial for the production of secondary plant products in which culantro is rich such as aromatic compound flavor and pharmaceuticals. Moreover, callus can be used for the production of somaclonal variants, which could improve plant characteristics.

2. MATERIALS AND METHODS

2.1. Source of explant

Seeds of Culantro plants (*Eryngium foetidum* L.), obtained from Miami, Florida, USA, were grown in a shadehouse. All experiments

were conducted, during 2000 and 2001, at the Genetic Engineering and Biotechnology Research Institute at Sadat City, University of Minufiya.

2.2. Preparation of root explants

Roots (approximately 30 to 40 mm in length) were excised from plantlets regenerated *in vitro* from leaf explants (Mohamed-Yasseen, 2001). Root explants were cut crosswise into approximately two equal sections proximal and distal or left intact.

2.3. Preparation of inflorescence-node and flower explants

Inflorescence-nodes (approximately 10 to 12 mm in length) and young immature flowers were washed with a detergent, rinsed with water and surface sterilized in 70% ethanol for 3-5 sec. Explants were rinsed with sterile distilled water then surface sterilized with 0.79% (v/v) sodium hypochlorite for 10 min. Explants were rinsed three times in sterile distilled water then placed on culture media.

2.4. Preparation of leaf explants

Leaves (approximately 50 to 60 mm in length) were excised from plantlets produced *in vitro*. Leaves were divided crosswise and only the proximal section was used in culture.

2.5. Culture *in vitro*

Root and leaf explants were placed on 25 ml of culture media contained in 118- or 177-ml baby food jars and sealed with clear plastic polypropylene lids (Sigma, Saint Louis, MO). Inflorescence-nodes and flowers were placed on 25 ml of culture media contained in 55-ml culture tubes and sealed with clear plastic polypropylene lids.

2.6. Composition of culture media

Culture media were composed of MS containing 30 g/l sucrose, 8 g/l agar and supplemented with appropriate growth regulators. Shoot-regeneration medium from root, inflorescence-node and flower explants was composed of MS medium alone or supplemented with 1, 2, 3, 4 or 5 mg/l BA with 0.5 mg/l NAA. Callus-induction medium was composed of MS medium containing 100 mg/l glutamine and 300 mg/l casine hydrolysate and supplemen-

ted with 0.5, 3, 6 or 10 mg/l 2,4-D, 3 mg/l picloram, 3 mg/l NAA, 3 mg/l NOA or 6 mg/l IAA. Shoot-induction medium from callus was composed of MS medium supplemented with 0.5 mg/l BA. Root-induction medium was composed of MS medium supplemented with 0.1 mg/l NAA.

2.7. Media and culture conditions

Media pH was adjusted to 5.7 with 1N KOH after adding growth regulators but before adding agar. Growth regulators were added before sterilization in an autoclave at 121 °C and 98 kPa for 20 min. Cultures were maintained at 26 ± 2°C and under an 18 hr photoperiod (cool white fluorescent light, 40 $umol.m^{-2}.s^{-1}$). Only callus cultures were maintained in the dark.

2.8. Rooting and transfer to soil

All produced shoots were separated and transferred into root-induction medium. Rooted shoots were planted in 165 cm^3 plastic pots filled with autoclaved commercial potting soil (Agro Mix no. 2; Conard Fafard, Springfield, Mass) and covered with glass beakers for 7-10 days.

2.9. Experimental design

All experiments were conducted using a completely randomized design. Twenty replicates were used in each treatment and each experiment was repeated at least twice. Treatment means were evaluated using Duncan's Multiple Range Test (Duncan, 1955).

3. RESULTS AND DISCUSSION

Root, inflorescence-node and flower explants produced multiple shoots on shoot-induction medium after seven to eight weeks from culture. Callus was produced from leaf explants on callus induction-medium after twelve to fourteen weeks from culture. Type of explants and growth regulators influenced morphogenesis in shoot-regeneration and callus formation.

3.1. Morphogenesis of root explants

Root explants produced shoots on MS medium free from growth regulators (Table 1). Number of produced shoots, shoot length, root number and percentage of shoot regeneration were higher from root explants cultured on MS medium free from growth regulators. The majority of regenerated shoots were produced from the proximal end of root explant (Fig. 1a). However, few numbers of shoots were produced from the middle of root explant but not from the root tip.

Table (1):Effect of BA and NAA concentration on shoot regeneration from culantro root explants after eight weeks from culture.

Supplements) (mg/l	shoot/explant	shoot length (mm)	root/explant)	shoot Regeneration %
None	2.0 a [z]	25.0 a	6.5 a	70.3
0.5 BA + 0.5 NAA	1.0 b	4.5 c	2.3 b	15.0
1 BA + 0.5 NAA	1.3 b	11.2 b	3.2 b	38.4
2 BA + 0.5 NAA	1.0 b	7. 2 c	0.4 c	32.6
3 BA + 0.5 NAA	0.5 c	2.8 d	0.9c	30.0
4 BA + 0.5 NAA	0.5 c	1.8 d	0.0 d	15.8
5 BA + 0.5 NAA	0.4 c	2.2 d	0.0 d	12.0

[z] Mean separation by Duncan's multiple range test at P = 0.05.

The effect of polarity was also observed when roots were cut transversally into distal and proximal sections. Shoot regeneration, shoot length and root number were higher from proximal sections (Table 2), suggesting that shoot regeneration occurred from older well-developed root explants.

Table (2): Effect of root explant position on shoot regeneration after eight weeks from culture. Explants were cultured on MS containing 1mg/l BA and 0.5 mg/l NAA.

Type of explant	% shoot regeneration	shoot length (mm)	root/explant
proximal	39.5	11.7 a [z]	3.4 a
distal	15.5	2.3 b	0.0 b

[z] Mean separation by Duncan's multiple range test at P = 0.05.

Although the number of regenerated shoots did not exceed two shoots per root explant, this method has several advantages. It is possible to use this method for clonal propagation because regenerated shoots were produced directly from root explants cultured on MS medium free from growth regulators and without callus formation. Moreover, this method offers a continuous system for having a permanent and sterile source of explants since rooted shoots can be used, after root harvest, to reproduce roots and so on.

3.2. Morphogenesis of inflorescence-node and flower explants

Inflorescence-node produced multiple shoots in MS medium free from growth regulators (Table 3). The numbers of produced shoots, shoot length, root number and percentage of shoot regeneration were influenced by the concentration of BA and NAA. The highest number of regenerated shoots occurred on MS medium containing 4 mg/l BA and 0.5 mg/l NAA. Shoot length decreased with increasing BA concentrations while the highest percentage of shoot regeneration was obtained on MS medium containing 1 mg/l BA and 0.5 mg/l NAA.

Table (3): Effect of BA and NAA concentrations on shoot regeneration from culantro node after eight weeks from culture.

Supplements (Mg/l)	shoot/explant	shoot length (mm)	root/explant	% shoot regeneration
0.0	2.4 c [z]	28.8 ab	3.2 c	45
0.5 BA + 0.5 NAA	2.3 c	34.0 a	2.8 c	58
1 BA + 0.5 NAA	3.0 b	30.3 a	7.8 a	80
2 BA + 0.5 NAA	3.2 b	30.1 a	6.5 a	70
3 BA + 0.5 NAA	3.2 b	22.0 c	5.2 b	65
4 BA + 0.5 NAA	4.0 a	1 6.5 c	3.4 c	60
5 BA + 0.5 NAA	3.6 ab	12.0 d	2.3 c	60

[z] Mean separation by Duncan's multiple range test at P = 0.05.

Flower explants produced multiple shoots (Fig. 1b), (average 2.6 $\pm$ 0.8 shoots/flower). Shoot regeneration was forty percent which may be due to the fact that flower explants turned into black colour

(Fig. 1b) and produced dark exudates into culture medium. Incubation of flower explants in solutions containing antioxidants such as PVP, ascorbic acid or cysteine, for 10 min, prior transfer to culture medium or incorporating activated charcoal into culture medium, did not improve shoot regeneration.

3.3. Callus production and shoot induction

Callus formation occurred from leaf explants cultured on MS medium containing 2,4-D, picloram, NAA and NOA. Leaf explants did not produce callus on MS medium containing IAA and only root formation occurred on this medium. Callus produced on 0.5, 3 or 6 mg/l 2,4-D was friable yellowish (Fig. 1c), while callus produced on picloram, NAA and NOA was whitish. Callus weight was high on MS medium containing 0.5 mg/l 2,4-D. However, callus weight decreased with increasing 2,4-D concentration. Callus was also produced from different explants of culantro such as shoot tip, bracts, root explants and flower explants cultured on MS medium containing 3 mg/l 2,4-D. Produced friable callus was transferred into shoot-induction medium for shoot formation. Culantro is an aromatic and medicinal plant, therefore, callus production from culantro could be used for secondary plant products such as aromatic compounds, flavors and pharmaceuticals.

Table (4): Effect of different auxins on callus production from culantro leaf explant.

Auxin	Callus weight (g)	% callus formation	Type of callus and morphogenesis
0.5 mg/l 2,4-D	9.7 a	90	friable yellowish callus, 5% root formation
3 mg/l 2,4-D	4.8 b	90	friable yellowish callus
6 mg/l 2,4-D	4.1 b	80	friable yellowish callus
10 mg/l 2,4-D	2.8 c	50	friable brownish callus
3 mg/l Picloram	5.3 b	50	friable callus
3 mg/l NAA	10.2 a	60	friable callus, 20% root formation
3 mg/l NOA	2.9 c	30	friable callus, 10% root formation
6 mg/l IAA	0 d	0	no callus formation, 60% root formation

*Mean separation by Duncan's multiple range test at $P = 0.05$.

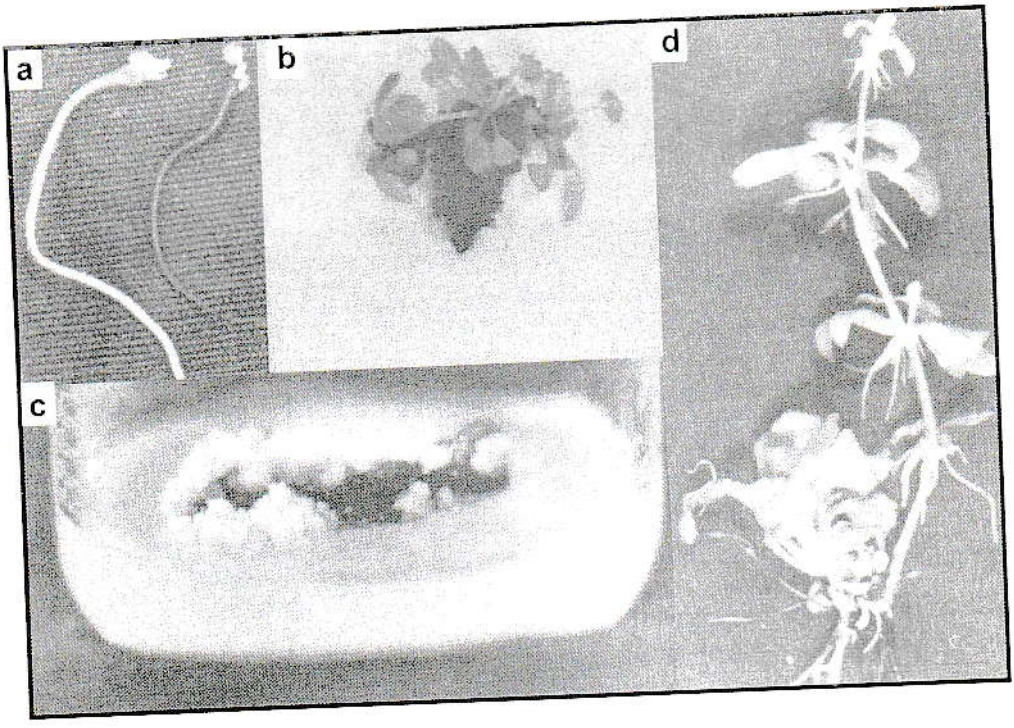

Fig 1. Morphogenesis of different explants of culantro. (a) Shoot regeneration from root explants. (b) Shoot regeneration from flower explant, notice the dark colour of flower explant. (c) Friable callus formation from leaf explant. (d) Shoot elongation and rooting prior transfer to soil, notice the flower formation *in vitro*.

3.4. Rooting and transfer to soil.

Shoots produced from both types of process, direct shoot formation and through callus formation, were transferred into root-induction medium. Virtually, all shoots produced roots and some formed flowers *in vitro* (Fig. 1 d). Rooted shoots were transferred to soil and produced normal phenotypic fertile plants.

In conclusion, this paper describes two protocols for plant formation from culantro. The first protocol describes direct shoot formation from root, inflorescence-node and flower explants. The second protocol describes shoot formation from callus. Both protocols offer the basis for breeding and cloning of culantro.

4. REFERENCES

Arockiasamy S. and Ignacimuthu S.(1998). Plant regeneration from mature leaves and roots of *Eryngium foetidum* L., a food flavouring agent. Curr. Sci. 75: 664-666.

Duncan D.B. (1955). Multiple range and multiple F tests. Biometrics 11:1-42.

Garcia M.D., Saenz M.T., Gomez M.A. and Fernandez M.A. (1999). Topical antiinflammatory activity of phytosterols isolated from *Eryngium foetidum* on chronic and acute inflammation models. Phytotherapy Res.13:78-80.

Ignacimuthu S., Arockiasamy S., Antonysamy M. and Ravichandran P.(1999). Plant regeneration through somatic embryogenesis from mature leaf explants of *Eryngium foetidum*, a condiment. Plant Cell Tiss. Org.Cult. 56:131-137.

Kuebel K.R. and Tucker A. (1988). Vietnamese culinary herbs in the United States. Economic Bot. 42:413-419.

Leclercq P.A., Dung N.X., Lo V.N., Toanh N.V., Nguyen X.D., Vu N.L. and Nguyen V.T. (1992). Composition of the essential oil of *Eryngium foetidum* L. from Vietnam. J. Ess. Oil Res 4: 423-424.

Mohamed-Yasseen Y. (1994). *In vitro* flowering and clonal propagation of culantro (*Eryngium foetidum* L.) from nodal explant. Plant Growth Regul. Soc. Amer. 22:21.

Mohamed-Yasseen Y. (2001). Micropropagation of culantro (*Eryngium foetidum* L.). *In Vitro Cel.* Dev. Biol. (In press).

Murashige T. and Skoog F.(1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Pino J.A., Rosado A. and Fuentes V. (1997).Composition of the leaf oil of *Eryngium foetidum* L. from Cuba. J. Ess. Oil Res. 9: 467-468.

Saenz M.T., Fernandez, M.A. and M.D. Garcia. (1997). Antiinflammatory and analgesic properties from leaves of *Eryngium foetidum* L. (Apiaceae). Pytotherapy Res. 11:380-383.

Wong K.C., Feng M.C., Sam T.W. and Tan G.L. (1994).Composition of the leaf and root oils of *Eryngium foetidum* L. J. Essen. Oil Res. 6: 369-374.

إكثار نبات الكيلانترو في المزارع النسيجية

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ملخص

نبات الكيلانترو نبات عشبى يستخدم في التوابل وله إستخدامات طبية. تم إكثار نبات الكيلانترو في المزارع النسيجية من الجذور وعقد النورات و الأزهار والأوراق. تمت زراعة الجذور و عقد النورات و الأزهار على بيئة موراشيج وسكوج بدون منظمات النمو أومحتوية على ١أو ٢ أو ٣ أو ٤ أو ٥ ملليجرام/لتر بنزيل أدينين و٠,٥ ملليجرام/لتر نفثالين حمض الخليك كما تمت زراعة الأوراق على بيئة موراشيج وسكوج المحتوية على٣٠٠ ملليجرام/لتر كازين هيدروليزات و ١٠٠ مليجرام/لتر جلوتامين ومحتوية على ٠,٥ أو ٣ أو ٦ أو١٠ ملليجرام/لتر توفوردى أو ٣ ملليجرام/لتر بيكلورام أو٦ ملليجرام/لتر نفثالين حمض الخليك أو٣ ملليجرام/لتر نفثوكسى حمض الخليك أو٦ ملليجرام/لتر إندول حمض الخليك. تم تكوين الأفرع من الجذورو عقد النورات و الأزهار وكان لطبيعةالجزء النباتى وتركيز البنزيل أدينين ونفثالين حمض الخليك تأثيرا" كبيرآ على عدد الأفرع وطولها وعدد الجذور المتكونة. تم تكوين الكالوس من الأوراق المنزرعة على2,4-D و البيكلورام ونفثالين حمض الخليك و نفثوكسى حمض الخليك ولكن إندول حمض الخليك شجع تكوين الجذور و لم يشجع تكوين كالوس.تم إنتاج نباتات من الكالوس بعد زراعته على بيئة موراشيج وسكوج المحتوية على ٠,٥ ملليجرام/لتر بنزيل أدينين. نقلت الأفرع المتكونة الى بيئة ميراشيج وسكوج المحتوية على ٠,١ ملليجرام/لتر نفثالين حمض الخليك لتشجيع تكوين الجذور و نقلت النباتات التي كونت جذورالى الصوبة وتم الحصول على نباتات مزهرة.

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