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PLANT REGENERATION FROM LEAF BLADE, PEDICLE AND SHOOT TIP OF CULANTRO (*Eryngium foetidum* L.), A MEDICINAL PLANT

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ABSTRACT

Culantro is an aromatic plant, which is used as condiment and medicinal plant. A procedure for shoot regeneration from leaf blade, pedicle and shoot tip explants, is described. Leaf blade, pedicle and shoot tip explants were cultured on Murashige and Skoog medium (MS) alone or supplemented with 0.5, 1, 2, 3, 4, 5, 6 mg/l benzyladenine (BA) with 0.5 mg/l naphthaleneacetic acid (NAA). Shoot regeneration from the proximal third section of leaf blade was higher than shoot regeneration from the middle and distal sections. Shoot regeneration, shoot length and roots number were influenced by BA and NAA concentrations. Shoot regeneration from quartered shoot tip was higher than shoot regeneration from decapitated or intact shoot tip. Produced shoots were transferred into root-induction medium and rooted shoots were successfully established in soil.

Keywords: Tissue culture, herb, medicinal plant, coriander substitute, herbs and spices.

Abbreviations: BA, benzyladenine; MS, Murashige & Skoog's (1962) medium; NAA - naphthaleneacetic acid.

INTRODUCTION

Culantro (*Eryngium foetidum* L., Apiaceae) is a biennial herb indigenous to continental Tropical America and the West Indies. The plant has a tap root with long, evenly branched roots and oblanceolate leaves arranged spirally around the short thick stem. Although widely used throughout the Caribbean, Latin America, and the Far East, culantro is unfamiliar in Egypt and relatively unknown in North America and many other parts of the world. It is often mistaken and misnamed for its close relative cilantro or coriander (*Coriandrum sativum* L.). Some of its common names descriptive of the plant include: spiny or serrated coriander, recao and fit weed (Morton, 1976, Ramcharan, 1999).

Culantro is used as a substitute for coriander in many tropical and subtropical countries (Kuebel and Tucker, 1988). The plant is used in traditional medicines for treating several diseases (Wong, 1976; Ramcharan, 1999) such as chills, vomiting, diarrhea and convulsions (Honeychurch,1980). The leaves and roots are boiled and the water is used for pneumonia, flu, diabetes, constipation, and malaria fever. The root can be used for scorpion stings and to alleviate stomach pains (Mahabir 1991) Saenz *et al.* (1997) and Garcia *et al.* (1999) were able to isolate antiinflammatory and analgesic compounds from leaves of culantro.

Plant regeneration from leaf petiole and inflorescence node was first described by Mohamed-Yasseen (1994) using thidiazuron Plant regeneration was also reported using BA (Arockiasamy and Ignacimuthu, 1998; Ignacimuthu *et al.* 1999). This paper described a protocol with high

frequency for direct shoot regeneration from leaf blade and pedicle and shoot proliferation from the shoot tip explants.

MATERIALS AND METHODS

Source of explants. Seeds of culantro (*Eryngium foetidum* L.), obtained from Miami, Florida, were grown on a shadehouse. All experiments were conducted from 2000 to 2002 at the Genetic Engineering and Biotechnology Research Institute at Sadat City, University of Minufiya.

Preparation of leaf blade and pedicle explants. Young leaves (approximately 40 to 60 mm in length) were excised from culantro plants. Leaf blade was cut transversally into three equal sections (approximately 10 to 15 mm in length), as proximal, middle and distal. Pedicle explants (approximately 8 to 12 mm in length) were excised carefully without perceptible buds. Leaf blade and pedicle explants were washed with detergent, rinsed with water and surface sterilized in 70 % ethanol for 3-5 sec. Explants were then rinsed with sterile distilled water and surface sterilized with 0.8 % (v/v) sodium hypochlorite for 10 min. Explants were rinsed three times in sterile distilled water and placed on culture media.

Effect of position of leaf blade explant. Proximal, middle and distal leaf blade sections were used in an experiment carried out to study the effect of explant position within its source on shoot regeneration. Explants were cultured on MS media containing 30 g/l sucrose, 8 g/l agar, 1.5 mg/l BA and 0.5 mg/l NAA.

Effect of BA concentrations and NAA on shoot regeneration from leaf blade explants. Only proximal leaf blade explants were used in this experiment conducted to study the effect of BA and NAA concentrations on shoot regeneration. Proximal leaf blade explants were cultured on MS containing 30 g/l sucrose, 8 g/l agar and supplemented with 0.0, 0.5, 1, 2, 3, or 4 mg/l BA and 0.0 or 0.5 mg/l NAA.

Effect of BA and NAA on shoot regeneration from pedicle explants.

Pedicle explants were cultured on MS containing 30 g/l sucrose, 8 g/l agar and supplemented with 0.0, 0.5, 1, 2, 3, 4 or 5 mg/l BA and 0.0 or 0.5 mg/l NAA.

Shoot prloiferation from shoot tip explants.

Source of shoot tip explants. Shoot tip explants were procured from plantlets regenerated from leaf blade explants cultured on 1mg/l BA and 0.5 mg/l NAA. Shoots were transferred into shoot elongation medium composed of MS containing 30 g/l sucrose and 8 g/l agar for 6-8 weeks.

Preparation of shoot tip explants. Shoots reaching 80 to 120 mm in length, were used. Leaves were detached from the short thick stem (approximately 4 mm in length) and branched roots were removed. Trimmed shoots were then, quartered longitudinally into four explants, decapitated or left intact for comparison. Shoot tip explants were cultured on MS containing 30 g/l sucrose, 8 g/l agar without growth regulators or supplemented with 0.0, 0.5, 1, 2, 3, 4, 5 or 6 mg/l BA and 0.0 or 0.5 mg/l NAA.

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

Leaf blade, pedicle and shoot tip explants were placed on 35 ml of culture media contained in 150- or 300-ml jars and sealed with clear plastic polypropylene lids Cultures were maintained under an 18 hr photoperiod (cool white fluorescent light, 40 $\mu mol.m^{-2}.s^{-1}$) and 26 ± 2°C

Rooting and transfer to soil. Produced shoots were separated and cultured for eight weeks on root-induction medium composed of MS containing 30 g/l sucrose, 8 g/l agar with 0.1 mg/l NAA. 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.

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. Data were evaluated by analysis of variance (Duncan, 1955).

RESULTS AND DISCUSSION

Morphogenesis of leaf blade explants. Shoots were regenerated from leaf blade explants after eight weeks from culture. Leaf blade explants produced small darkish callus with very short shoots and green nodular structures within the first four weeks (Fig. 1a). Multiple shoots arised upon transfer to fresh medium (Fig. 1b). Morphogenesis was influenced by the position of leaf blade explant within its source. Shoot regeneration, shoot length and roots number were high from the proximal section and decreased gradually upward (Table 1). The position of explant within its source was reported to influence morphogenesis (Mohamed-Yasseen *et al.* 1994a). Therefore, subsequent experiments were conducted using proximal section of leaf blade explant. Shoot regeneration, shoot length, roots number and percentage of explants producing shoots were influenced by BA and NAA concentrations (Table 2). Shoot regeneration, shoot length roots number and percentage of explants producing shoots increased when culture medium was containing 1mg/l BA and 0.5 mg/l NAA, then decreased with increasing BA concentration.

Morphogenesis of pedicle. Pedicle produced shoots in MS alone or containing BA and NAA (Fig.1 c), however the frequency of shoot regeneration was higher when BA and NAA were added to culture media (Table 3). Shoots number increased with increasing BA concentration from 0.0 to 2 mg/l BA. The highest number of regenerated shoots (3.4 shoot per explant) was obtained in MS containing 2 mg/l BA. Shoot number and frequency of shoot regeneration decreased by increasing BA concentration over 2 mg/l BA. Shoot length and roots number were affected by BA and NAA concentrations. Shoot length and roots number decreased by increasing BA concentration above 2 mg/l BA.

Table(1): Effect of position of leaf blade explant within its source on shoot regeneration on MS containing 1.5 mg/l BA and 0.5 mg/l NAA.

Position of explant within leaf	shoot/explant	shoot length (mm)	root/explant	% explants producing shoot
proximal	2.0 a[z]	16.5 a	3.9 a	75
middle	1.2 b	5.2 b	0.4 b	40
distal	0.1 c	5.0 b	0.0 c	15

[z] Measen paration by Duncan's multiple range test at P = 0.05.

Table(2): Effect of BA and NAA on morphogenesis of culantro leaf blade explant after eight weeks from culture. Culture medium was composed of MS alone or containing 0.5 mg/l NAA and different concentrations of BA.

BA	shoot/explant	shoot length (mm)	root/explant	% explants producing shoots
0.0	0.3 c[z]	10.6 b	0.9 b	7
0.5 mg/l	1.5 b	7.5 b	1.2 b	16
1 mg/l	2.5 a	20.0 a	8.1a	80
2 mg/l	1.8 ab	7.8 b	3.7 b	70
3 mg/l	1.4 b	3.0 c	0.5 c	40
4 mg/l	1.3 b	3.0 c	0.5 c	40

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

Table(3): Effect of BA and NAA on morphogenesis of pedicle explants after eight weeks from culture. Culture medium was composed of MS alone or containing 0.5 mg/l NAA and different concentrations of BA.

BA	shoot/explant	shoot length (mm)	root/explant	% explants producing shoots
0.0	0.4 d[z]	19.4 a	6.1 b	22
1 mg/l	2.4 ab	16.7 a	5.2 b	100
2 mg/l	3.4 a	21.2 a	12.5 a	100
3 mg/l	2.0 bc	15.2 b	3.1 c	80
4 mg/l	1.3 c	8.2 c	1.6 c	80
5 mg/l	1.0 cd	3.1 d	0.5 d	65

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

Morphogenesis of shoot tip. Intact and quarter shoots cultured on MS devoid of BA did not proliferate (Table, 4), while decapitated shoots produced multiple shoots (Fig. d). Intact shoots did not produce multiple shoots when BA concentration increased to 3 mg/l, while decapitated shoots and quarter shoots produced multiple shoots. Intact shoots produced few number of shoots when BA was 4 to 6 mg/l which may be due to apical dominance. The highest number of shoot formation from one single shoot was obtained from quartered shoots cultured on MS containing 4 to 6 mg/l BA. Using the technique of quartering shoot tip, it was possible to obtain over 16 shoots from one single shoot. The presence of BA and elimination of apical dominance by decapitating or quartering shoot tip were necessary for high shoot proliferation. Similar results were obtained with shoot tip of several Allium species (Mohamed-Yasseen *et al.*, 1994b; 1996).

Rooting and Transfer to soil. Produced shoots from all type of explants formed roots and elongated in MS medium supplemented with 0.1 mg/l NAA. All shoots produced multiple roots easily in root-induction medium. Rooted shoots were transferred into soil and virtually all plantlets were successfully established in soil and normal phenotypic flowering plants were obtained (Fig. 1e).

Table(4): Effect of BA and NAA on morphogenesis of shoot tip explnat after eight weeks from culture. Culture medium was composed of MS alone or containing 0.5 mg/l NAA and different concentrations of BA.

BA (mg/l)	Intact shoot		decapitated shoot		quarter shoot	
	shoot/explant	length (mm)	shoot/explant	length (mm)	shoot/explant	length (mm)
0.0	1.0 b[2]	94.0 a	2.1 c	81.0 a	1.0 d	91.0 a
0 5	1.0 b	85.0 a	2.2 c	50.5 b	1.8 c	76.0 a
1.0	1.0 b	72.0 a	2.1 c	58.0 b	2.2 c	53.0 b
2.0	1.0 b	60.0 b	3.0 b	38 0 c	2.5 c	32.0 c
3.0	1.0 b	60.0 b	3.0 b	28.0 c	3.4 b	25.0 c
4.0	1.2 b	55.0 b	3.5 a	19.5 c	4.5 a	20 5 c
5.0	1.5 a	43.3 c	3.6 a	20.0 c	4.6 a	15.0 d
6.0	1.6 a	40.5 c	3 0 b	13.0 d	4.0 b	15.0 d

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

This paper described two protocols for plant production from culantro using leaf blade, pedicle and shoot tip. The first protocol described plant regeneration through adventitious shoots from leaf blade and pedicle. The second protocol describes shoot proliferation from shoot tip through quartering or decapitation of shoot tip. Both protocols offer the basis for the purposes of breeding and cloning of culantro.

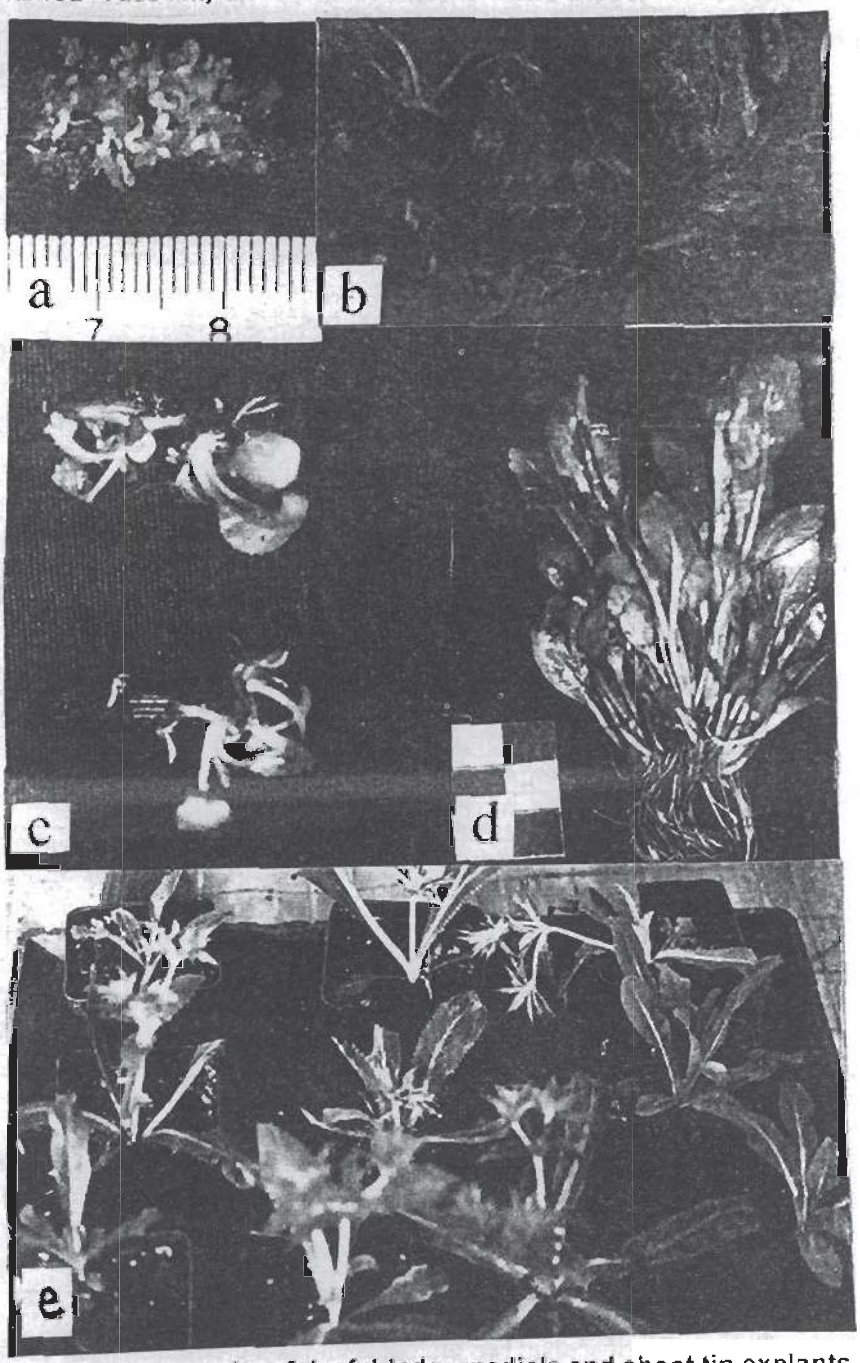

Fig 1: Morphogenesis of leaf blade, pedicle and shoot tip explants of culantro. (a). Callus formation and shoot initiation from leaf blade. (b). Shoot regeneration from leaf blade. (c). Shoot regenerqation from pedicle. (d). Shoot proliferation from decapitated shoot. (e). Culantro plants flowering in soil after acclimatization.

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إكثار نبات الكيلانترو في المزارع النسيجية من الأوراق والحامل الزهرى والبراعم الطرفية

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نبات الكيلانترو نبات عشبى له إستخدامات طبية عديدة كما يستخدم فى التوابل. تم إكثار نبات الكيلانترو في المزارع النسيجية من الأورق والحامل الزهرى والبراعم الطرفية . تمت زراعة الأورق والساق الزهرية والبراعم على بيئة ميراشيج وسكوج بدون منظمات النمو أومحتوية على ١أو ٢ أو ٣ أو ٤ أو٥ او ٦ملليجرام/لتر بنزيل أدينين و٥, ملليجرام/لتر نفثالين حمض الخليك وقد وجد ان الأجزاء السفلى من الورقة يعطى عدد كبير من البراعم اكثر من الأجزاء الوسطى والعليا من الورقة. كما تم الحصول على نباتات من الساق الزهرية. وقد وجد ان قطع البرعم الطرفى طوليا" الى اربعة أجزاء أدى الى زيادة عدد النباتات . كما وجد ان عدد الأفرع المتكونة وكذلك طول الأفرع وعدد الجذور المتكونة عليها قد تأثر بتركيز منظمات النمو فى البيئة. نقلت الأفرع المتكونة الى بيئة ميراشيج وسكوج المحتوية على ١, ملليجرام/لتر نفثالين حمض الخليك لتشجيع تكوين الجذور و نقلت النباتات التى كونت جذور الى الصوبة وتم الحصول على نباتات مزهرة.