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SHOOT PROLIFERATION AND PLANT REGENERATION FROM GIANT TARO (*Alocasia macrorrhiza* L.) SCHOTT AND ENDL)

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ABSTRACT

Alocasia macrorrhiza is an ornamental plant with decorative foliage. A procedure for propagation of Alocasia from shoot tip, axillary buds, stem pith, root and leaf petiole explants is described. Shoot tip and axillary buds were excised from mature plants and cultured on Murashige and Skoog medium (MS) supplemented with 8.8 μM benzyladenine (BA) and 0.5 μM naphthaleneacetic acid (NAA) and incubated for eight weeks. Produced shoots were quartered, or left intact and cultured on MS alone or supplemented with 2.2, 4.4, 8.8, 13.2, 17.6, 22.0, 26.4, 30.8, 35.2 μM BA with 0.5 μM NAA. Shoot regeneration was obtained from stem pith explants cultured on MS alone or supplemented with 0.9, 2.2, 4.4, 8.8, 13.3, 17.6, 22.0 μM BA with 0.5 μM NAA. Callus was produced from shoot tip, leaf, root and stem pith explants cultured on MS supplemented with different concentration from 2,4-D, picloram or NAA. Shoot regeneration from callus occurred upon transfer into MS containing 2.2 μM BA. Produced shoots were rooted, acclimatized, and normal plants were successfully established in soil.

INTRODUCTION

Alocasia macrorrhiza is an ornamental evergreen monocotyledonous plant with decorative foliage, appertains to the family Araceae. The plant has also other names such as giant taro and giant Alocasia (Foliaki *et al.*, 1990; Wen *et al.*, 1997b). Some cultivars are cultivated to a limited extent in India and some of the Pacific Islands for food consumption (Jackson, 1999; Le *et al.*, 1999). The nutritional value of Alocasia was extensively studied (Bradbury and Singh, 1986a, b; Bradbury and Holloway, 1988: Singh and Bradbury, 1988). It is also pointed out that this crop could be grown for animal feed (Ravindran and Rajaguru, 1985: Maratos, 1996) as its leaves and stem have high protein content (Foliaki *et al.*, 1990). In addition, Alocasia leaves are excellent source for carotenoids (Wen *et al.*, 1997b).

However, Alocasia, like most tuber crops, is vulnerable to viral diseases (Thankappan, 1993) which hinder its breeding programs. Moreover, several cases of toxicity were recorded due to exposure plant exudates or consumption of Alocasia (Chan *et al.*, 1995). The presence of adverse chemical factors which cause acridity and toxicity was investigated (Hammer *et al.*, 1989; Wen *et al.*, 1997a, Lin *et al.*, 1998; Paull *et al.*, 1999). The toxicity and acridity of Alocasia are believed to be due to a combination of calcium oxalate (Bradbury and Nixon, 1998; Holloway *et al.*, 1989), trypsin inhibitor, sapotoxin and other unidentified chemical irritants (Chan *et al.*, 1995). The presence of toxic chemicals reduced greatly the exploitation of Alocasia plant as ornamental plant and affects its palatability.

Establishment of an efficient system for shoot proliferation from pre-existing buds (Mohamed-Yasseen and Splittstoesser, 1995) and plant regeneration from adventitious buds and callus (Mohamed-Yasseen *et al.*, 1995) has the potential for producing free disease plants, mass propagation and improving characteristics of Alocasia. There is no available information about tissue culture of *Alocasia macrorrhiza*. This paper describes an efficient system for shoot formation, callus induction and plant regeneration using explants derived from shoot tip, stem pith, root and leaf explants.

MATERIALS AND METHODS

Source of initial explants. Shoot tip and axillary buds were removed from mature plants. All experiments were conducted, during a period from 2000 and 2002, at the Genetic Engineering and Biotechnology Research Institute at Sadat City, University of Minufiya.

Explant preparation. Shoot tip and axillary buds (approximately 5-12 mm, in length) were washed with detergent and rinsed with water. Explants were surface sterilized in 70% ethanol for 1 min, rinsed with distilled water then surface sterilized with 0.79% (v/v) sodium hypochlorite for 15 min. Explants were rinsed three times in sterile distilled water prior culture *in vitro*.

Shoot production. Explants were placed on 25 ml of culture media contained in 55-ml culture tubes and sealed with plastic polypropylene lids (Sigma, Saint Louis, MO). Culture medium was composed of Murashige and Skoog (1962) medium (MS) containing 30 g/l sucrose, 7 g/l agar (Agar-Agar/ Gum agar, Sigma) supplemented with 8.8 µM BA and 0.5 µM NAA. Produced shoots were transferred after eight weeks into fresh medium dispensed into 300 ml glass jar capped with clear plastic polypropylene lids, for further proliferation. Shoot-proliferation medium was composed of MS supplemented with 4.4 µM BA with 0.5 µM NAA.

Produced shoots were cultured on MS-free growth regulators, for additional eight weeks, for root formation. Plantlets produced from MS-free growth regulators were then utilized in this paper as a source of explants (shoot tip, stem pith, root and leaf explants) which were used to study the effect of growth regulators on shoot proliferation, callus formation and plant regeneration.

Effect of BA and NAA on shoot proliferation from Alocasia shoot tip.

Shoot tips (approximately 5-12 mm, in length) were left intact or quartered and cultured on MS containing 30 g/l sucrose, 8 g/l agar and supplemented with 0.0, 2.2, 4.4, 8.8, 13.2, 17.6, 22.0, 26.4, 30.8, 35.2 µM BA with or without 0.5 µM NAA.

Effect of BA and NAA on shoot regeneration from stem pith explant.

Stem pith explants (approximately, 8-12 mm, in length) were excised and cultured on MS supplemented with 0.0, 0.9, 2.2, 4.4, 8.8, 13.3, 17.6, 22.0 µM BA with or without 0.5 µM NAA.

Callus induction from shoot tip, root, leaf and stem pith explants.

Shoot tip, root, leaf and stem pith explants were excised from plantlets produced *in vitro* and cultured on MS containing 30 g/l sucrose and 2g/l gelrite (Phytagel, gelrite, Sigma) and supplemented with appropriate auxin. Intact and quartered shoot tip was prepared as above and cultured on callus induction medium composed of MS supplemented 6.8, 13.6, 27.1µM 2,4-D or 12.4 µM picloram. Root explants (approximately 15 to 30 mm, in length) were cultured on callus induction medium composed of MS supplemented with 6.8, 13.6, 27.1 and 45.2 µM 2,4-D. Leaf explants were cut into leaf blade and leaf petiole. Leaf petiole was divided into proximal and distal section (approximately 8-12 mm, in length) and leaf blade was cut into 3- 6 segments. Leaf explants were cultured on callus induction medium composed of MS supplemented with 2.3, 6.8, 13.6, 27.1, 45.2 µM 2,4-D or 12.4 µM picloram. Stem pith explants, prepared as described above, were cultured on callus induction medium composed of MS supplemented with 2.3, 6.8, 13.6, 27.1, 45.2 µM 2,4-D , 12.4 µM picloram or 16.2 µM NAA.

All explants were maintained in the dark and produced callus was transferred to light into shoot-induction medium composed of MS containing 30g/l sucrose, 7 g/l agar and supplemented with 2.2 µM BA.

Media and culture conditions. Media pH was adjusted to 5.7 with 1N KOH after adding growth regulators but before adding gelling agent. Growth regulators were added before sterilization in an autoclave at 121 °C and 98 KPa for 20 min. Shoot formation cultures were maintained under an 18 hr photoperiod (cool white fluorescent light, 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 28 °C., while callus induction cultures were maintained in the dark.

Transfer to soil. All produced shoots were separated and transferred for rooting and elongation into MS containing 30 g/l sucrose and 7 g/l agar. Rooted shoots were planted in 165 cm^3 plastic pots filled with autoclaved commercial potting soil (Agro Mix no. 2; Conard Fafard, Springfield, Mass).

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).

RESULTS AND DISCUSSION

Shoot proliferation from Alocasia shoot tip was considerably influenced by concentration of BA and NAA. Number of produced shoots, shoot length and number of roots were affected with concentrations of BA and NAA (Table 1). The highest number of shoot proliferation was obtained on MS supplemented with 30.8 µM BA and 0.5 µM NAA. This latter suggested that high concentration of BA was necessary to obtain high number of shoot proliferation. Shoot length and number of roots per explants were shown to be reduced with increasing BA concentration. Quartering shoot tip is easy to operate on Alocasia shoot tip and has the advantage of obtaining four explants instead of one single explant.

Table 1. Effect of BA and NAA concentrations on shoot proliferation from *Alocasia macrorrhiza* after eight weeks from culture.

Supplement	type of explant	shoot/explant	shoot length (mm)	root/explant
0.0	intact-shoot	1.0 e[z]	90 a	18.9 a
0.0	quarter-shoot	1.0 e	80 a	16.7 a
2.2 µM BA + 0.5 µM NAA	intact-shoot	1.0 e	88 a	15.2 a
2.2 µM BA + 0.5 µM NAA	quarter-shoot	1.2 e	71 b	15.9 a
4.4 µM BA + 0.5 µM NAA	intact-shoot	1.2 e	80 ab	13.5 a
4.4 µM BA + 0.5 µM NAA	quarter-shoot	1.7 e	62c	10.5 a
8.8 µM BA + 0.5 µM NAA	intact-shoot	1.5 de	74 b	12.9 a
8.8 µM BA + 0.5 µM NAA	quarter-shoot	1.9 de	74 b	10.9 a
13.2 µM BA + 0.5 µM NAA	intact-shoot	2.5 d	65 c	9.7 ab
13.2 µM BA + 0.5 µM NAA	quarter-shoot	2.0 de	52 de	8.1 ab
17.6 µM BA + 0.5 µM NAA	intact-shoot	3.0 cd	60 cd	8.3 ab
22.0 µM BA + 0.5 µM NAA	intact-shoot	4.3 c	50 de	7.2 c
26.4 µM BA + 0.5 µM NAA	intact-shoot	5.6 b	45 fe	6.5 c
30.8 µM BA + 0.5 µM NAA	intact-shoot	6.2 a	38 f	5.2 d
35.2 µM BA + 0.5 µM NAA	intact-shoot	5.0 b	30 f	2.4 d

[z] Means having the same letters are not significantly different according to Duncan's multiple range test, P = 0.05.

Dividing shoot tip in some Allium spp increased the number of produced shoots per explant due to eradication of apical dominance and removal of anatomical barriers, thereby permitting axillary buds to grow (Mohamed-Yasseen *et al.*, 1994). This method was beneficial for producing high number of shoots from several monocotyledonous plants (Mohamed-Yasseen, 1999). Quartering shoot tip in Alocasia did not increase the number of produced shoots per explant. Shoot formation was rather higher from intact shoot of Alocasia cultured on MS supplemented with 13.2 µM BA and 0.5 µM NAA. Moreover, shoots produced from quarter-shoot were thinner and leaves were smaller than those produced from intact-shoot.

Shoot regeneration from stem pith was influenced by the concentration of BA and NAA (Table, 2). Number of shoots per explant was increased with increasing BA concentration then decreased afterward.

The highest number of regenerated shoots was attained on MS supplemented with 8.8 µM BA and 0.5 µM NAA. Shoot length and number of roots per explant decreased with increasing BA concentration. Stem pith is a new category of explants for shoot production through adventitious shoot formation. However, the source of stem pith explant is limited since each shoot usually provide one single stem pith explant.

The effect of different auxins on callus formation from different explants was investigated (Tables, 3,4,5,6). Callus was produced from diverse Alocasia explants such as shoot tip, root, leaf and stem pit explants cultured on MS supplemented with 2,4-D, picloram or NAA. Friable callus formation from intact shoot and quartered tip was accomplished on MS supplemented with 2,4-D (Fig. 1a) or picloram (Table, 3). Callus produced from shoot tip on 2,4-D was yellowish and smaller than that produced on picloram.

Table 2. Effect of BA and NAA on shoot regeneration from stem pith of *Alocasia macrorrhiza* after eight weeks from culture.

Supplements	shoot/explant	shoot length (mm)	root/explant
0.0	0.8 e	55 a	8.3 a
0.9 µM BA	1.2 d	65 a	6.4 a
2.2 µM BA + 0.5 µM NAA	2.5 c	63 a	5.2 a
4.4 µM BA + 0.5 µM NAA	3.3 b	45 b	5.0 a b
8.8 µM BA + 0.5 µM NAA	4.0 a	40 b	4.6 b
13.3 µM BA + 0.5 µM NAA	3.5 b	30 c	3.2 c
17.6 µM BA + 0.5 µM NAA	2.8 c	24 d	2.6 c
22 µM BA + 0.5 µM NAA	1.5 d	12 d	0.9 d

[z] **Means having the same letters are not significantly different according to Duncan's multiple range test, P = 0.05.**

Table 3. Effect of 2,4-D and picloram on callus formation from whole or quarter shoot tip of *Alocasia macrorrhiza* after twelve weeks from culture.

Auxin (mg/l)	type of explant	callus weight (g/explant)	type of morphogenesis
6.8 µM 2,4-D	whole shoot	1.44 b	few yellowish friable callus
13.6µM 2,4-D	whole shoot	1.30 b	few yellowish friable callus
27.1 µM 2,4-D	whole shoot	0.60 c	few yellowish friable callus
6.8 µM 2,4-D	quarter shoot	1.2 b	few yellowish friable callus
13.6µM 2,4-D	quarter shoot	0.83 bc	few yellowish friable callus
27.1 µM 2,4-D	quarter shoot	0.65 c	few yellowish friable callus
12.4 µM picloram	quarter shoot	4.5 a	whitish friable callus

Means having the same letters are not significantly different according to Duncan's multiple range test, P = 0.05.

There were no significant differences between callus weights produced from intact or quarter shoot tip. Callus weight decreased with increasing concentration of 2,4-D from 6.8 µM to 27.1µM.

Friable callus was produced from root and leaf explants on MS supplemented with 2,4-D or picloram (Table, 4,5). It is generally noticed that callus size produced from root and leaf explants becomes yellowish, more friable and granular with embryo like structures (Fig. 1b) with increasing concentration of 2,4-D from 2.3 µM to 13.6 µM 2,4-D. Callus was produced from proximal and distal section of leaf. Wile leaf blade did not produce callus on all 2,4-D, picloram or NAA.

Fig. 1. Typical callus formation from different explants of Alocasia and plant regeneration *in vitro*. a: typical friable callus production from shoot tip. b: typical friable callus production from root explants. c: typical friable callus from stem pith explants, d: typical shoot regeneration from Alocasia callus after rooting and prior transfer to soil.

Table 4. Effect of 2,4-D, picloram and NAA on callus formation from root explants of *Alocasia macrorrhiza* after twelve weeks from culture.

Auxin (mg/l)	callus weight (g/g explant)	callus formation%	morphogenesis
6.8 µM 2,4-D	2.6 a	100	friable callus
13.6µM 2,4-D	1.8 b	100	friable callus
27.1 µM 2,4-D	1.6 bc	100	friable callus
45.2 µM 2,4-D	1.2 c	65	friable callus
12.4 µM picloram	3.2 a	70	slightly friable callus
16.2 µM NAA	0.8 d	10	friable callus

[z] Means having the same letters are not significantly different according to Duncan's multiple range test, P = 0.05.

Table 5. Effect of 2,4-D, picloram and NAA on callus formation from leaf petiole of *Alocasia macrorrhiza* after twelve weeks from culture.

Auxin (mg/l)	leaf explant	callus weight (g/g explant)	callus formation%	morphogenesis
2.3 µM 2,4-D	proximal	3.6 b	7	yellowish friable callus
6.8 µM 2,4-D	proximal	3.8 b	31	yellowish friable callus
13.6µM 2,4-D	proximal	4.3 a	34	yellowish friable callus
27.1 µM 2,4-D	proximal	3.1b	15	yellowish friable callus
45.2 µM 2,4-D	proximal	2.8 c	12	yellowish friable callus
12.4 µM picloram	proximal	2.9 c	18	whitish friable callus
6.8 µM 2,4-D	distal	3.0 b	17	yellowish friable callus
16.2 µM NAA	proximal	0.0	0.0	no callus formation

[z] Means having the same letters are not significantly different according to Duncan's multiple range test, P = 0.05.

Friable callus was produced from stem pith explants (Table 6) on MS supplemented with 2,4-D, picloram or NAA (Fig. 1c). Callus weight and percentage of callus formation decreased with increasing concentration of 2,4-D. Sporadic shoots were directly produced on MS supplemented with low concentration of 2,4-D (2.3 µM 2,4-D) or supplemented 16.2 µM NAA, while high concentration of 2,4-D (45.2 µM 2,4-D) caused necrosis and stem pith explants died ultimately.

Table 6. Effect of different auxins on callus formation from stem pith of *Alocasia macrorrhiza* after eight weeks from culture.

Supplements	callus weight (g/g)	callus %	Type of morphogenesis
2.3 µM 2,4-D	2.8 a	100	friable callus with sporadic shoots
6.8 µM 2.4-D	1.8 b	100	friable callus formation
13.6 µM 2,4-D	1.4 c	100	small friable callus
27.1 µM 2,4-D	1.1 c	80	small friable callus
45.2µM 2,4-D	0.0 d	0.0	no callus formation and necrosis
12.4 µM picloram	2.1b	100	friable callus
16.2 µM NAA	2.0 b	100	friable callus with sporadic shoots and roots

Means having the same letters are not significantly different according to Duncan's multiple range test, P = 0.05.

Multiple shoots were regenerated from callus derived from root, leaf petiole and stem pith explants and occasionally from shoot tip callus. Direct shoot formation from shoot tip or from stem pith explants is faster than shoot

regeneration from callus. However, shoot regeneration from callus is more valuable for breeding purposes. Produced shoots were rooted (Fig. 1d) and normal plants were obtained in soil.

In conclusion, this paper, described two methods for shoot and plantlets production from several explants; shoot tip, stem pith explants, root and leaf explants. The first method demonstrated direct shoot formation which was accomplished through shoot proliferation from shoot tip and shoot regeneration from stem pith explants. The second method described shoot regeneration from callus which was achieved from callus derived from shoot tip, root, leaf and stem pith explants. Both methods offer the basis for mass propagation, production of free disease plants and improving plant characteristics.

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إكثار نبات الألوكاسيا في المزارع النسيجية

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تم إكثار نبات الألوكاسيا في المزارع النسيجية بزراعة القمة النامية على بيئة ميراشيج وسكوج الخالية من منظمات النمو اوالمحتوية على ٢،٢أو ٤،٤ أو ٨،٨ أو١٣،٢ أو١٧،٦ أو٢٢ أو٢٦،٤أو ٣٠،٨ أو ٣٥،٢ ميكرومول بنزيل أدينين و٥، ميكرومول نفثالين حمض الخليك.كما تم إستخدام الأنسجة النخاعية للساق في الإكثار وذلك بزراعتها على بيئة ميراشيج وسكوج بدون منظمات النمو أو محتوية على ٠،٩ أو٢،٢ أو ٤،٤ أو ٨،٨ أو١٣،٢ أو١٧،٦ أو٢٢ميكرومول بنزيل أدينين و٥، ميكرومول نفثالين حمض الخليك. كما تم إنتاج نباتات من الكالوس الذي تكون من القمة النامية والجذوروالأوراق و الأنسجة النخاعية للساق المنزرعة على بيئة ميراشيج وسكوج محتوية على تركيزات مختلفة من توفوردي أو بيكلورام أو نفثالين حمض الخليك وذلك بزراعة الكالوس على بيئة ميراشيج وسكوج المحتوية على ٢،٢ ميكرومول بنزيل أدينين. وقد أمكن الحصول على نباتات من الأجزاء النباتية المختلفة مباشرة أو من الكالوس المتكون. وكان لطبيعة الجزء النباتي وتركيز منظمات النمو تأثير كبير على عدد الأفرع وطولها وعدد الجذور المتكونة. نقلت الأفرع المتكونة الى بيئة ميراشيج وسكوج الخالية من منظمات النمو لتشجيع تكوين الجذور ونقلت النباتات التي كونت جذورا الى الصوبة وتم الحصول على نباتات مشابهة للأباء.