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In vitro CALLUS FORMATION AND PLANT REGENERATION FROM SEVERAL ONION (*Allium cepa* L.) EXPLANTS

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ABSTRACT

Immature inflorescences and young seedlings of onion were used as sources of explants for callus formation and plant regeneration *in vitro*. Inflorescence explants were cultured on Murashige and Skoog's (1962) medium (MS) containing 0.0, 0.3, 0.6 or 1 mg/l of benzyladenine (BA), kinetin or isopentenyladenine (2iP) with 0.01 mg/l naphthaleneacetic acid (NAA). Florets were cultured on MS medium containing 1.5 mg/l BA. Callus was induced from inflorescence explants and florets on callus-induction medium composed of MS containing 2 mg/l 2,4 ichlorophenoxyacetic acid (2,4-D) , 0.5 mg/l NAA, 0.5mg/l kinetin, 200 mg/l casine hydrolysate (CH), 200 mg/l glutamine and 5 mg/l silver nitrate. Callus was produced from onion seedlings which were germinated on callus-induction medium and from roots which were excised from young seedlings that were germinated on MS medium. All produced callus was transferred into shoot-regeneration medium composed of MS containing 0.3 mg/l BA. These shoots produced roots in MS medium containing 0.1 mg/l indolebutyric acid (IBA). Bulb induction was investigated using MS medium containing 30, 60 or 90 g/l sucrose; 20 mg/l salicylic acid (SA); 5 mg/l jasmonic acid (JA) and 1000 mg/l CH. Rooted shoots and produced bulbs were transferred into soil and normal phenotypic plants were successfully established in soil.

Key words: *bulb-induction , inflorescence, propagation, root, salicylic acid, seedlings.*

1. INTRODUCTION

Onion (*Allium cepa* L.) is considered one of the most important vegetable crops which is cultivated all over the world (Yamaguchi, 1983). Egypt is one of the leading countries in onion production, most of the production is exported. Tissue culture has the potential to improve plant characteristics through callus formation and plant regeneration.

Onion inflorescences are excellent materials for plant regeneration, since they are easier to surface sterilize compared to under-ground parts (Mohamed-Yasseen, 1999). Dunstan and Short (1979) were the first to use inflorescence of onion in tissue culture. Mohamed-Yasseen *et al.*, (1993, 1995) produced multiple shoots from 'Sweet Spanish'using thidiazuron. This paper describes the effect of three different cytokinins, BA, kinetin and 2iP, on organogenesis from inflorescence explants obtained from 'Red Behiri', an Egyptian cultivar. In addition, it investigates callus induction from inflorescence, roots and seedlings as well as plant regeneration from produced callus.

2. MATERIALS AND METHODS

2.1. Source of explants

Onion seeds of the Egyptian cv. Red Behiri were obtained from the Horticultural Research Center at Giza. Onion plants were grown in a shadehouse at the Genetic Engineering and Biotechnology Research Institute, Sadat City, Minufiya. Immature inflorescences were collected in April and May, 1999, 2000 and 2001. All experiments were conducted at the above mentioned institute.

2.2. Explant preparation

Immature inflorescences were surface-sterilized with 0.79% (v/v) sodium hypochlorite, with two drops of Tween, for 15-20 min then rinsed three times in sterile distilled water. Each inflorescence was trimmed about 6-8 mm above the peduncle stem and divided longitudinally into four equal sections, herein called explants, prior

culture *in vitro*. For callus formation from florets, they were excised from immature inflorescence with approximately 0.3 mm of pedicel prior culture on callus-induction medium.

Onion seeds were surface sterilized with 0.1% (w/v) mercury chloride, with two drops of Tween, for 10-12 min then rinsed in sterile distilled water. Seeds were, thereafter, surface-sterilized with 0.79% (v/v) sodium hypochlorite for 15-20 min and rinsed three times in sterile distilled water.

2.3. Culture *in vitro*

Inflorescence explants were placed on 25 ml of culture media in 55-ml culture tubes and sealed with clear plastic polypropylene lids (Sigma, Saint Louis, MO). Produced shoots were then separated and placed on 25 ml of culture in 150-or 300-ml jars and sealed with clear plastic polypropylene lids. For callus formation, all explants were cultured on callus-induction medium in 150-or 300-ml jars and sealed with clear plastic polypropylene lids.

2.4. Direct shoot formation from inflorescence explants

Shoot-induction medium was composed of MS medium supplemented with 30 g/l sucrose, 8 g/l agar (Bacto-agar, Difco, Fisher, Chicago, IL) and supplemented with 0.0, 0.3, 0.6, 1.0 mg/l BA with 0.01 mg/l NAA; 03, 0.6, 1.0 mg/l kinetin with 0.01 mg/l NAA or 0.3, 0.6, 1.0 mg/l 2iP with 0.01 mg/l NAA. Explants were also cultured on MS supplemented with 90 g/l sucrose alone or with 5g/l activated charcoal (AC). Shoots were produced from inflorescence explants after six to eight weeks from culture. Produced shoots were then separated and used for bulb induction, rooting or further proliferation. Shoot proliferation medium was composed of MS medium supplemented with 30 g/l sucrose, 8 g/l agar and 0.5 mg/l BA.

2.5. Two-step shoot formation from inflorescence explants

Inflorescence-explants were first cultured on callus-induction medium composed of MS medium supplemented with 30 g/l sucrose, 2 g/l gelrite, 2 mg/l 2,4-D, 0.5 mg/l NAA, 0.5mg/l kinetin, 200 mg/l CH, 200 mg/l glutamine and 5 mg/l silver nitrate. Explants were maintained in the dark on callus-induction medium for 10-12 weeks. Produced callus was then transferred into shoot-induction medium

composed of MS supplemented with 30 g/l sucrose, 8 g/l agar and 0.2 mg/l BA.

2.6. Direct shoot formation from florets

Florets were cultured on MS medium supplemented with 30 g/l sucrose, 2 g/l gelrite and 1.5 mg/l BA.

2.7. Two-step shoot formation from florets

Florets were cultured on callus-induction medium. Callus was maintained for 12-14 weeks in the dark prior transfer to shoot-induction medium.

2.8. Callus induction and shoot regeneration from seedlings

2.8.1. Direct callus formation from seedlings

Onion seeds were germinated directly on callus-induction medium.

2.8.2. Callus induction from seedling roots

Onion seeds were germinated in 55-ml culture tubes, each containing 25 ml of MS medium supplemented with 30 g/l sucrose, and 8 g/l agar. Roots were excised from 5-7 week old seedlings. Roots were cut into small segments, approximately 20-30 mm in length, and cultured on callus induction-medium.

2.9. Bulb induction

Bulb-induction medium was composed of MS supplemented with 30, 60, 90 g/l sucrose, 1 mg/l BA, 20 mg/l salicylic acid, 5 mg/l jasmonic acid, 60 g/l sucrose and 1 mg/l BA, 60 g/l sucrose and 20 mg/l salicylic acid, 60 g/l sucrose and 5 mg/l jasmonic acid and 60 g/l sucrose and 1000 mg/l casein hydrolysate.

2.10. Media and culture conditions

Media pH was adjusted to 5.7 with potassium chloride 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 under an 18 hr photoperiod (cool white fluorescent light, 40 umol m-2 s-1) and 28 °C except callus cultures which were maintained in the dark.

2.11. Rooting and transfer to soil

Produced shoots were rooted in root-induction medium composed of MS supplemented with 30 g/l sucrose, 8 g/l agar and 1 mg/l IAA.

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. Produced bulbs can be transferred directly to soil without acclimatization or may be stored under 4 °C for several months until needed.

2.12. Experimental design

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

3. RESULTS AND DISCUSSION

Shoot proliferation occurred from onion inflorescence after culture on MS medium without any additives or by the addition of cytokinine. The numbers of produced shoots, shoot length and percentage of explants producing shoots were generally influenced by cytokinine type and concentration (Table 1). All explants produced shoots even in the absence of cytokinine. The number of shoots increased by increasing cytokinine concentration. The highest number of shoots was obtained with 0.6 and 1 mg/l BA followed by 1 mg/l kinetin. Number of shoots was medium with 0.3 mg/l BA, 0.6 mg/l kinetin and 0.3 mg/l kinetin. Shoots reached its lowest number on media containing 2iP or free of growth regulators. Increasing sugar concentration to 90 g/l considerably reduced shoot length. This might be due to the increase of osmotic pressure of nutrient medium. The highest percentage of explants producing shoots occurred with 0.6 and 1 mg/l BA and 1 mg/l kinetin. All concentations of 2iP and low concentrations of kinetin gave a low percentage of explants that produced shoots. High sugar concentration seemed to increase the frequency of shoot proliferation from inflorescence explants.

Shoots were also produced from inflorescence through a two-step process (Table 2). Inflorescence-explants were first cultured on

callus-induction medium for 10-12 weeks prior transfer to the shoot-induction medium. Inflorescence-callus produced high number of shoots (average3 ± 0.8 SD shoots/explant) upon transfer to shoot-induction medium. However, the frequency of shoot regeneration from inflorescence-callus was twenty percent. Shoot regeneration from inflorescence derived-callus was a slow procedure and had less frequency than the direct shoot proliferation. Nevertheless, shoot formation from callus, has several beneficial applications such as production of somaclonal variations and selection of plants with desirable traits.

Table (1): Effect of type and concentration of cytokinine on shoot proliferation from inflorescence of onion cv. Red Behiri.

Medium composition	Shoot number	Shoot length (cm)	% Explants producing shoots
MS alone	1.4 e [z]	10.3 a	50
MS + 90 g/l sucrose	2.0 cd	6.3 bc	70
MS + 90 g/l sucrose + 5 mg/l AC	2.0 cd	4.5 cd	70
MS + 0.3 mg/l BA + 0.01 mg/l NAA	3.2 bc	8.2 a	90
MS + 0.6 mg/l BA + 0.01 mg/l NAA	4.6 a	7.5 ab	100
MS + 1mg/l BA + 0.01 mg/l NAA	4.8 a	7.1 ab	100
MS + 0.3 mg/l kinetin + 0.01 mg/l NAA	3.0 bc	8.8 a	80
MS + 0.6 mg/l kinetin + 0.01 mg/l NAA	3.1 bc	8.1 a	90
MS + 1mg/l kinetin + 0.01 mg/l NAA	4.2 ab	7.2 ab	100
MS + 0.3 mg/l 2iP + 0.01 mg/l NAA	1.8 de	8.9 a	70
MS + 0.6 mg/l 2iP + 0.01 mg/l NAA	2.2 cd	8.5 a	70
MS + 1 mg/l 2iP + 0.01 mg/l NAA	2.6 cd	8.2 a	80

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

Shoots were produced from florets through callus formation on BA or 2,4-D containing medium. Florets produced green callus and shoot primordia after 8-10 weeks from culture under light prior transfer to shoot-induction medium. Low percentage of explants (1-3%) produced shoots (Table 2). Although the frequency of shoot formation was very low, flower culture had the potential for production of haploid plants (Campion *et al.*, 1992) through gynogenesis. Moreover, each inflorescence of onion usually contains high numbers of flowers (30-45

flower per inflorescence).

Table (2): Callus formation and shoot regeneration from inflorescence, florets and seedlings of onion cv. Red Behiri. Data are presented as the mean (± SE) based on twenty explants per treatment.

Type of explant	Culture medium	Shoot/explant	% Explants producing shoots
Inflorescence	callus induction medium	3.0 ± 0.8	20
Florets	BA containing medium	1.5 ± 0.2	3
Florets	callus induction medium	1.0 ± 0.3	1
Seedlings	callus induction medium	1.2 ± 0.2	5
Roots	callus induction medium	0.0	0.0

Callus was produced from onion seedlings germinated directly on callus-induction medium and from root explants. Seedlings produced pale yellow callus, from root and basal stem areas, after 12-14 weeks from culture in the dark.Produced callus was transferred into shoot-induction medium. Percentage of callus formation from germinated seedlings on callus induction medium was 100 percent. Produced callus was nodular and of an embryo-like structure, however, only five percent of the callus produced shoots. Callus was produced from root explants after 12-14 weeks from culture in the dark. Percentage of callus formation from root explants was 15 and shoot regeneration did not occur from root callus.

Bulb formation from onion shoots was affected by several factors (Table 3). Bulb weight reached its maximum when sucrose concentration was 60 and 90 g/l. The addition of BA did not seem to have a significant effect on bulb weight, while SA and JA reduced bulb weight. SA and JA inhibited shoot and root length which could explain the reduced bulb weight. CH did not increase bulb weight but increased shoot and root length. High sucrose concentration is known to induce bulb formation (Mohamed-Yasseen and Splittstoesser, 1992). SA and JA were reported to induce bulb formation in garlic (Zel *et al.*, 1997; Xiong *et al.*, 1999), however they showed no stimulatory effect on onion bulb formation.

Table (3): Effect of sucrose, BA, SA, JA and CH on onion bulb induction.

Medium composition	Bulb weight (mg)	Shoot length (mm)	Root length (mm)
MS alone	140 c	108 a	78 a
MS + 60 g/l sucrose	445 b	37 c	42 c
MS + 90 g/l sucrose	622 a	22 c	30 d
MS + 1 mg/l BA	78 d	28 c	35 d
MS + 20 mg/l SA	92 d	26 c	15 e
MS + 5 mg/l JA	83 d	24 c	12 e
MS + 60 g/l sucrose + 1 mg/l BA	330 b	32 c	28 d
MS + 60 g/l sucrose + 20 mg/l SA	125 c	38 c	16 e
MS + 60 g/l sucrose + 5 mg/l JA	87 d	27c	12 e
MS + 60 g/l sucrose + 1000 mg/l CH	338 b	75 b	57 b

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

Produced shoots from inflorescences, florets and seedling callus were transferred to rooting medium and virtually all shoots produced roots. Rooted shoots were transferred to soil and normal phenotypic fertile plants were produced. Some shoots were also transferred to bulb-induction medium. Bulbs can be stored for several months or transferred directly to the soil.

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تكوين كالوس ونباتات من عدة أجزاء نباتية من البصل فى المزارع النسيجية

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ملخص

تم إستخدام نورة البصل غير مكتملة النضج وكذلك البادرات لإنتاج كالوس ونباتات فى المزارع النسيجية. تم زراعة أجزاء النورة فى بيئة محتوية على تركيزات مختلفة من البنزيل أدنين أو الكينيتن او الأيزوبنتيل أدينين. تم إنتاج الكالوس في بيئة محتوية على ٢مليجرام/لتر من توفوردى و٥, مليجرام/لتر من نفثالين حمض الخليك و٥, مليجرام/لتر كينيتين و ٢٠٠ مليجرام/لتر كازين هيدروليزات و ٢٠٠ مليجرام/لتر جلوتامين و ٥ مليجرام/لتر نترات فضة. تم الحصول على كالوس من بذور البصل المزروعة على بيئة الكالوس وتم نقل

الكالوس الى بيئة محتوية على ٢, مليجرام/لتر بنزيل أدنين لتشـــجبع تكويـــن الأفرع وتم تشجيع تكوين الجذور على بيئة محتوية على١, مليجرام/لتر إندول حمض البيوتريك. درس تـــأثير الســـكروز وحمـــض السلســـليك وحمـــض الجاسمونيك والكازين هيدروليزات على تكوين الأبصال.نقلت النباتات التـــى كونت جذور و كذلك التى كونت أبصال الى الصوبة وتـــم الحصـــول علـــى نباتات مزهرة.

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