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Research paper

Micropropagation of Gerbera (*Gerbera jamesonii* Bollus) Using Capitulum Explants

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Abstract

Three separate experiments were conducted to study the effect of the cytokine benzyl amino purine (BAP) concentration (0, 2, 4, 6 and 10 mg l^{-1}) and medium status (solid with agar versus liquid with cotton support); number of capitulum sections (2, 4, 8, 16 sections) on *in vitro* shoot regeneration on capitulum explants of the gerbera cultivar "Evergreen" and indole-3-butyric acid (IBA) concentration (0, 0.5, 1, 2 mg l^{-1}) on *in vitro* rooting of shoots using half MS salt strength. Data were collected on percentage of responding explants, number of shoots/explant, rooting percentage, number of roots per shoot and root length. The highest percentage of responding explants (86.6 %) and highest number of shoots/explant (4.77 shoots) were recorded by the solid medium supplemented with 4 mg l^{-1} BAP. The solid medium gave significantly higher number of shoots/explant (2.37) than the liquid one (1.53). There was no shoot formation in BAP- free medium. Cutting the capitulum into 8 sections resulted in the highest percentage (76.6%) of responding explants and highest number of shoots per explant (6.55 shoots). There was no significant difference between treatments in percentage of rooted shoots. The highest rooting percentage (86.6%) resulted from the treatment 2 mg l^{-1} IBA and the lowest one (66.6 %) was given by the control. The treatment 2 mg l^{-1} IBA resulted in, significantly, the highest number of roots per shoot (8.8) and the lowest number of roots (2.1) was given by the control. There was no significant difference between treatments in root length. The treatment 0.5 mgl^{-1} IBA gave the highest root length (20.7 mm). The lowest root length (12.2 mm) was given by the control.

Keywords: Micropropagation, *Gerbera jamesonii*, Capitulum explants, benzylaminopurine, Indole butyric acid, *In vitro* rooting.

الإكثار الدقيق للجيربرا (*Gerbera jamesonii* Bollus) باستخدام البراعم الزهرية كأجزاء نباتية منفصلة

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المستخلص

أجريت ثلاث تجارب منفصلة لدراسة تأثير تركيز السايتوكاينين بنزيل أمينو بيورينBAP (0،2، 4، 6، 10 ملجم/ لتر) و حالة الوسط الغذائي (صلب باستخدام الأجار مقارنة مع الوسط السائل باستخدام القطن كدعامة)، وعدد قطع البرعم الزهري (2،4،8،16) على تكوين الأفرع على البراعم الزهرية الصغيرة لصنف الجيربرا Evergreen خارج الجسم الحي وتركيز أندول حمض البيوترك IBA (0.0، 0.5، 1.0، 2.0 ملجم/لتر) على تجذير الأفرع خارج الجسم الحي باستخدام نصف تركيز أملاح وسط موراشيجي و اسكوق (MS). جمعت بيانات عن نسبة الأجزاء النباتية المنفصلة التي استجابت وعدد الأفرع بالجزء النباتي المنفصل ونسبة التجذير وعدد الجذور بالفرع وطول الجذر. أعلى نسبة (86.6 %) للأجزاء النباتية المنفصلة التي استجابت و أعلى عدد للأفرع بالجزء النباتي المنفصل (4.77 فرع) فقد سجلت في الوسط الصلب المضاف إليه 4 ملجم/لتر BAP. الوسط الصلب أعطى و بدرجة معنوية أعلى عدد للأفرع بالجزء النباتي المنفصل (2.37 فرع) من الوسط السائل (1.53 فرع). قطع البرعم الزهري إلى 8 أجزاء نتج عنه أعلى نسبة من الأجزاء النباتية المنفصلة التي إستجابت (76.6 %) وأعلى عدد للأفرع بالجزء النباتي المنفصل (6.55 فرع). لم يكن هنالك فرق معنوي بين المعاملات في نسبة التجذير لكن المعاملة 2 ملجم/لتر IBA أعطت أعلى نسبة تجذير (86.6 %) وأقل نسبة تجذير (66.6 %) أعطيت بواسطة الشاهد. المعاملة 2 ملجم/لتر IBA نتج عنها وبدرجة معنوية أعلى عدد للجذور بالفرع (8.8) وأقل عدد للجذور (2.1) أعطي بواسطة الشاهد. الفرق بين المعاملات في طول الجذر لم يكن معنوياً أعطت المعاملة 0.5 ملجم/لتر IBA أطول جذر (20.7 ملم) بينما أعطت معاملة الشاهد أقصر جذر (12.2 ملم).

كلمات مفتاحية: الاكثار الدقيق، الجيربرا، البرعم الزهري كجزء نباتي منفصل، بنزيل أمينو بيورينBAP، اندول حمض البيوترك، التجذير خارج الجسم الحي.

Introduction

Gerbera (*Gerbera jamesonii Bolus*), commonly known as Transvaal Daisy and Barberton Daisy is an important cut flower worldwide. It ranks fifth in the international cut flower trade. Gerbera is ideal for beds, borders, pots and rock garden. The flowers which are of various colors suit very well in different floral arrangements. The cut blooms also have a long vase life of about 7 to 8 days (Van Son, 2007).

Like other ornamental plants gerberas are produced exclusively for their aesthetic values. The commercial cultivars of gerberas are propagated through vegetative means by divisions of

clumps, but the multiplication by this method is too slow to be commercially viable (Kanwar and Kumar, 2008). Propagation through seed is not preferred as the plant exhibit heterozygosity and lack uniformity. Also, the improved semidouble and double cultivars do not set seed. Rapid multiplication could be accomplished through micropropagation technique (Van Son, 2007). Tissue culture procedure has been proven to be commercially practical in gerbera propagation. This method enables a million fold expansion per year of a desired plant (Murashige *et al.*, 1974).

Gerbera was micro propagated using various explants including shoot tips, floral buds, leaf, capitulum. Gerbera plantlets were produced from capitulum by various workers (Pierik *et al*.,1982; Modh *et al*., 2002; Tyagi and Kothari, 2004; Ray *et al.,* 2005; Mohammed and Ozzambak, 2007). The advantages of the capitulum method over shoot tip are the easier sterile isolation *in vitro* and it is also non-destructive, only inflorescences are used and no shoots are lost from the plant (Kanwar and Kumar, 2008). Pierik *et al*. (1975) obtained shoots from fully developed (mature) gerbera capitulum explants using a medium with 10 mg/l BAP. They rooted the resultant shoots *in vitro* in a medium containing 10 mg/l of either IBA or IAA and they obtained nearly 90 % rooting. Laliberte *et al*. (1985) reported that immature capitula 0.5 – 0.7 cm in diameter were up to 10 times as productive as fully developed ones. Radice and Marconi (1998) obtained shoots from fragments of young gerbera capitulum (diameter =1cm) explants using a medium with 2 mg/l BAP. They rooted the resultant shoots in MS medium containing 0.5 mg/l IBA and they obtained 70 – 100 % rooting of shoots. The use of liquid medium in tissue culture is often described as a means of reducing the cost of micro propagation (Alvard *et al*., 1993). The advantages include increased availability of water and dissolved substances to the explants and lower labour and production costs. Shoot production of *Rhododendron* was ten-fold higher in liquid medium than on agar-solidified medium (Douglas, 1984). Puchooa *et al*. (1999) compared the performance of *Nicotiana tabacum* leaf explants in MS medium either in liquid form (static, static with filter paper+glass beads as support, and agitated liquid medium) or solidified with Difco Bacto- agar and gelrite. They found significant differences between the supports used in terms of fresh weight, dry weight and number of shoots produced. Best response was obtained with liquid agitated medium. Macleod and Nowak (1990) found no differences in the regeneration capability of white clover using either agar solidified medium or liquid medium supported with small solid glass beads as matrix. According to the same authors, a 60% saving on media components can thus be made by substituting agar with beads. While some plants do not grow well in liquid medium others grow well in it (Pierik, 1987).

The objectives of this study was to examine the effects of BAP concentration, medium status (solid with agar versus liquid with cotton wool support), number of capitulum sections on shoot regeneration on capitulum explant and IBA concentration on *in vitro* rooting of shoots of the gerbera cultivar "Evergreen" in order to establish a protocol for micropropagation of this cultivar.

Materials and methods

This study was carried out at the tissue culture laboratory of the Date Palm Technology Company at Shambat, Khartoum North, Sudan.

Explant: For experiments one and two, young capitula (small flower buds) 0.5 – 1 cm in diameter were collected from greenhouse plants of the gerbera cultivar "Evergreen ”growing at a greenhouse belonging to the Central Trade Company (CTC), Khartoum North, Sudan. For sterilization, explants were first washed with running tap water for 15 minutes, then washed with detergent solution and rinsed with tap water. The explants were then taken to the laminar air flow cabinet where they were dipped in 70% ethanol for 10 seconds, followed by immersion in 15% commercial bleach (Clorox) to which was added 150 mgl^{-1} citric acid and 100 mgl^{-1} ascorbic acid as antioxidant solution with 2 drops /100 ml of tween 20 for 30 minutes with continuous shaking. They were then rinsed 3 times with autoclaved distilled water. After disinfestation each capitulum was divided and placed onto the medium.

Culture Media: For experiments one and two, the nutrient medium was composed of half MS (Murashige and skoog, 1962) inorganic salts and in mg/l: 10000 sucrose; 8000 agar; 80 adenine sulphate; 0.1 indole acetic acid (IAA). The pH of the medium was adjusted to 5.7 prior to agar addition using 0.1N HCl or NaOH. The medium was dispensed at 12.5 ml in 25×150 mm test tubes, plugged with aluminum foil and autoclaved at temperature of 121 ^{o}C and a pressure of 1.05 kg cm^{-2} for 15 minutes and left to cool in the culture room at temperature of 25±2^{o} C.

Culture conditions: The cultures were first placed in darkness for 2 weeks and subsequently placed under continuous florescent light at an intensity of 40.5 µmol m^{-2} s^{-1} and photoperiodic cycle of 16/8 light/dark hours and temperature of 25±2°C for another 6 weeks.

Experiment one: Effect of BAP concentration and medium status on shoot regeneration:

Five concentrations of BAP (0, 2, 4, 6, 10 mg l^{-1}) and two forms of medium (semi-solid form solidified with agar and liquid form supported with cotton wool) were tested as a two factor experiment. Each capitulum was divided into 4 segments and placed horizontally onto the medium that composed of half MS inorganic salts and in mg l^{-1}: 10.000 sucrose; 8000 agar; 80 adenine sulphate; 0.1 indole acetic acid (IAA).

Experiment two: Effect of number of capitulum sections on shoot regeneration: The capitulum was divided into 2,4,8,16 sections as treatments and cultured onto a nutrient medium composed of half MS inorganic salts and in mgl^{-1}: 10.000 sucrose; 8000 agar; 80 adenine sulphate; 0.1 indole acetic acid (IAA); 5 mgl^{-1} BAP.

Experiment three: Effect of IBA concentration on *in vitro* rooting of shoots: *In vitro* shoots resulting from experiments one and two were cultured in Magenta GA7 plastic culture vessels containing nutrient medium composed of half MS inorganic salts and in mg l^{-1}: 10.000 sucrose; 8000 agar; 80 adenine sulphate. IBA concentrations tested were 0, 0.5, 1, 2 mgl^{-1}.

Experimental design and statistical analysis

In experiment one, the five concentrations of BAP and the two forms of medium were combined as a two factor experiment in a completely randomized design with three replications. In experiments two and three treatments were arranged in a completely randomized design and replicated thrice as single factor experiments. Three explants represented an experimental unit. Statistical analysis was performed using SAS statistical software (SAS Inst. USA, V. 11, 2002). Mean separation was performed using Duncan's Multiple Range Test at 5% level of significance.

Parameters measured: Data were collected on percentage of responding explants (explants that form shoots), number of shoots/explant, rooting percentage, number of roots per shoot and root length.

Results and discussion

Experiment one: Effect of BAP concentration and medium status on shoot regeneration on capitulum explants

Percentage of responding explants

As shown in Table 1, the interaction between BAP concentration and medium status had a significant effect ($P \leq 0.05$) on percentage of responding explants. The highest percentage of responding explants (86.6 %) was recorded by solid medium supplemented with 4 mg l^{-1} BAP. BAP concentration had a significant effect ($P \leq 0.05$) on explant response. The highest percentage of responding explants (71.1 %) was shown at 4 mg l^{-1}. Medium status had no significant effect on explant response. However, the solid medium gave higher percentage of responding explants (45.9%) than the liquid one (33.3%). There was no explant response to the BAP- free medium.

Number of shoots/explant

Data presented in Table 2, showed that the interaction between BAP concentration and medium status had a significant effect ($P \leq 0.05$) on number of shoots/explant. The highest number of shoots/explant (4.77) was recorded by solid medium supplemented with 4 mg l^{-1} BAP. The concentration of BAP had a significant effect ($P \leq 0.05$) on number of shoots/explant. The highest number of shoots (3.72) was shown at 4 mg l^{-1}. The solid medium gave significantly higher number of shoots (2.37) than the liquid one (1.53). There was no shoot formation in BAP- free medium (Table 2). Using gerbera capitulum as explants, Van Son (2007) found that addition of 3 mgl^{-1} of BAP to MS medium resulted in maximum number of responding explants in "Arianna" variety, while addition of 5 mgl^{-1} BAP gave highest response in "Bonnie" variety, whereas, variety "Tobia" exhibited highest response on 10 mgl^{-1} BAP. Mohammed and Ozzambak (2007) obtained higher number of responding capitulum explants in the gerbera cultivar "Ameretto" at 7 and 10 mgl^{-1} BAP than at lower concentrations of 2 and 5 mgl^{-1} BAP. Pierik *et al.* (1975) found no response of gerbera capitulum explants to BAP- free medium. Laliberte *et al*. (1985) found maximum number of shoots on MS medium supplemented with 2 mgl^{-1} BAP from "Pastourelle" gerbera variety capitulum explant. Radice and Marconi (1998) also obtained axillary shoots from young capitulum of different gerbera cultivars on a medium that contained BAP at 2.0 mgl^{-1}. Van

Son (2007) obtained 7.40 shoots per explants on 3 mgl^{-1}BAP from "Ariaana" variety, 6.20 shoots on 5 mgl^{-1} BAP from "Bonnie" variety, whereas variety "Tobia" produced 5.40 shoots on 10 mgl^{-1} BAP. Pierik *et al*. (1975) used fully developed capitulum of gerbera as explants and reported that there was no shoot developed on cytokinin-free media and the optimum concentration of cytokinin was 10.0 mgl^{-1} BAP. These differences in capitulum explant response to shoot formation indicate that shoot formation on Gerbera capitulum explants depends on both cultivar and cytokinin level in the medium (Pierik *et al.*, 1982). Growth response in gerbera capitulum explant is said to be cultivar specific (Schiva *et al*., 1982; Pierik *et al*., 1982 and Harel *et al*., 1993) and every genotype has a specific range of optimum growth regulator concentration (Deepaja,1999). The differences noticed among different research workers could be attributed to genotypic differences and to the interaction effect of endogenous and exogenous growth regulators.

Plants differ in their response towards medium status either being solid or liquid. Pierik (1987) stated that some plants do not grow well in liquid medium others grow well in it. Working with several gerbera cultivars, Mohammed and Ozzambak (2007) showed that response towards medium status depends on the gerbera cultivar. Macleod and Nowak (1990) found no differences in the regeneration capability of white clover using either agar solidified medium or liquid medium supported with small solid glass beads as matrix. Shoot production of *Rhododendron* was found to be ten-fold higher in liquid medium than on agar-solidified medium (Douglas, 1984).

Table (1): Percentage of responding explants of the gerbera cultivar "Evergreen" as affected by BAP concentration and medium status two months after culture.

BAP concentrations (mgl^{-1})	Liquid medium (%)	Solid medium (%)	Mean
0	0.0 b	0.0 b	0.0 A
2	66.6 a	44.4 ab	55.5 AB
4	55.5 a	86.6 a	71.1 B
6	44.4 ab	66.6 ab	55.5 AB
10	0.0 b	44.4 ab	22.2 AB
Mean	**33.3 A**	**45.9 A**	

Means followed by the same letter "s" are not significantly different (P = 0.05) according to Duncan's Multiple Range Test.

Experiment two: Effect of number of capitulum sections on shoot regeneration on capitulum explants

Percentage of responding explants

Significantly (P≤ 0.05) highest response (76.6%) was obtained by cutting the capitulum into 8 sections followed by 4 sections and 16 sections. The lowest response (33.3%) was given by 2 sections.

Table (2): Number of shoots/explant of the gerbera cultivar "Evergreen" as affected by BAP concentration and medium status two months after culture.

BAP concentrations (mgl^{-1})	Liquid media (%)	Solid media (%)	Mean
0.0	0.0 a	0.0 a	0.0 A
2	1.91 ab	2.11 ab	2.05 ABC
4	2.66 ab	4.77 b	3.72 C
6	3.47 b	3.55ab	3.51 C
10	0.18 ab	2.48 ab	1.33 AB
Mean	1.53 A	2.37 B	

Means followed by the same letter "s" are not significantly different (P = 0.05) according to Duncan's Multiple Range Test.

Number of shoots/explant: As shown in Table 3, cutting the capitulum into 8 sections resulted in Significantly (P≤ 0.05) highest number of shoots/explant (6.55). Several workers used capitulum as explant in gerbera as it has remarkable advantage over shoot tip explant which costs the life of the plant from which it is taken (Tyagi and Kothari, 2004). Shoot development in capitulum explant might be due to the formation of meristematic tissues in segment of the immature flower heads (Mandal *et al.*, 2002). Shoot development from dormant buds situated in the axils of the bracts surrounding the receptacles of capitulum has also been reported by Pierik *et al.* (1975). Bhatia *et al.* (2012) divided immature gerbera capitulum into 4-8 sections and obtained 10 shoots/section in 11 weeks. Laliberte *et al*. (1985) divided immature gerbera capitulum (0.5- 0.7 cm in diameter) into 20 sections and obtained 12 shoots/section in 12 weeks. Mandal *et al.* (2002) divided immature gerbera capitulum into 12 pieces and obtained 5 shoots/piece. Genotypic differences might be responsible for such variation in results.

Table (3): Effect of number of capitulum sections on shoot formation in the gerbera cultivar "Evergreen "eight weeks after culture.

Number of capitulum sections	Percentage of responding explants (%)	Number of shoots/explant
2	33.3 a	2.25 a
4	66.6 ab	4.55 ab
8	76.6 b	6.55 b
16	66.6 ab	2.66 ab

Means followed by the same letter "s" in the same column are not significantly different (P = 0.05) according to Duncan's Multiple Range Test.

Experiment three: Effect of IBA concentration on *in vitro* rooting of shoots

Rooting percentage: As shown in Table 4, there was no significant difference between treatments in percentage of rooted shoots. However, the treatment 2 mg l^{-1} IBA gave the highest rooting percentage (86.6%) followed by the treatments 0.5 and 1 mg l^{-1} IBA (76.6%). The lowest percentage was given by the control (66.6%).

Number of roots per shoot: The highest value was significantly ($P \leq 0.05$) recorded by the treatment 2 mgl^{-1} (8.8 roots) and the lowest value (2.1 roots) was given by the control (Table 4).

Root length: There was no significant difference between treatments in root length. The treatment 0.5 mgl^{-1} IBA gave the highest root length (20.7 mm). The lowest root length (12.2 mm) was given by the control (Table 4). Different plant species and cultivars show different responses to IBA concentration in *in vitro* rooting. In sugarcane (*Saccharum officinarum*) the best *in vitro* rooting of microshoots was at 5.0 mg/l (Baksha *et al*., 2003). Cos *et al.* (2004) studied the optimal *in vitro* conditions to induce explant rooting of the 'Mayor'® peach-almond hybrid. They compared different IBA concentrations (1, 1.5, 2, 2.5 and 3 mg/l). The best inclusion in the culture media was 2 mg/l with a 73,6% success rate. In many previous studies gerbera shoots were rooted *in vitro* using different concentrations of different auxins with high success. Radice and Marconi (1998) obtained 70-100% rooting of gerbera shoots on MS medium supplemented with 0.5 mgl^{-1} IBA. Working with the gerbera cultivar Ameretto, Mohammed and Ozzambak (2007) obtained 100% rooting of shoots on MS medium supplemented with IBA at 0.5, 1, 2 mg/l and the highest number of roots per shoot was obtained at 1 mg/l. Pierik *et al*. (1975) obtained nearly 90% rooted gerbera shoots using IAA or IBA at a concentration of 10 mgl^{-1}. Such variation in results might be attributed to genotypic differences. As a conclusion, culturing young capitula (0.5 – 1 cm in diameter), cut into 8 sections onto half MS medium solidified with agar and supplemented with 4 mg l^{-1} BAP and *in vitro* rooting of the resultant shoots onto half MS medium fortified with 2 mg l^{-1} IBA can be suggested as a protocol for micropropagation of the gerbera cultivar "Evergreen".

Table (4): Effect of IBA concentration on root formation on shoots of the gerbera cultivar "Evergreen" two months after culture.

IBA concentration (mg l^{-1})	Percentage of rooted shoots (%)	No of roots per shoot	Root length (mm)
0	66.6 a	2.1 a	12.2 a
0.5	76.6 a	7.1ab	20.7 a
1	76.6 a	5.7 ab	19.1 a
2	86.6 a	8.8 b	17.2 a

Means followed by the same letter "s" in the same column are not significantly different (P = 0.05) according to Duncan's Multiple Range Test.

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