In *vitro* regeneration of gerbera

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Abstract

A system for *in vitro* regeneration of adventitious shoots from callus was developed in current work. Callus was formed from *in vitro* juvenile leaf explants of the Gerbera "*Gerbera jamesonii*" on 58 different MS-based media containing different concentration and combinations of plant growth regulators, i.e., BAP, Kin, Zeatin, NAA, IAA and ABA. Two different shoot formation media were evaluated, both contain 2 mg/l NAA but they have different concentration of BAP (2.0 and 4 mg/l). The highest shoot regeneration frequency was 36.6 % which was obtained on induction medium containing 2 mg/l BAP and 0.25 mg/l ABA and shoot formation medium containing 2 mg/l of each of BAP and NAA. Regenerated shoots were successfully rooted on MS medium containing 40 µg/l NAA.

Keywords: Gerbera jamesonii, regeneration, BAP (benzylaminopurine), NAA (a-naphthalenacetic acid), IAA (indole acetic acid)

Zusammenfassung

***In vitro* Regeneration von Gerbera**

Ein System für die *in vitro* Regeneration von „*Gerbera jamesonii*" wird vorgestellt.

Es wurden 58 verschiedene Kombinationen von Medien und Wachstumsreglern geprüft. Das beste Regenerationsergebnis wurde bei mit 36,6 % auf einem Induktionsmedium mit 2 mg/l BAP und 0.25 mg/l ABA erzielt.

Schlüsselwörter: Gerbera jamesonii, Regeneration, BAP (Benzylaminopurin), NAA (a-Naphthalenessigsäure), IAA (Indolessigsäure)

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1 Introduction

Gerbera (*Gerbera jamesonii*) is a valuable ornamental species grown as a potted plant as well as for cut flowers. It is considered one of the most economically important cut flower in the world. Egypt with its warm weather can be considered a good location for cut flowers production. Furthermore, the Europe market has increased its demand of gerbera in the recent years. Therefore, the gerbera growers are looking for a novel gerbera generation with excellent characters of color, stem length, petal shape, vase life and others to expand their market in Europe. Plant breeding, besides producing better crop plants, has also focused on species that are attractive and have aesthetically valuable characteristics. Traditionally, breeding of ornamental plants has been based mainly on continuous crossing and selection for combining commercially important characteristics into élite genotypes (Elomaa et al., 1993). However, two or three cycles of inbreeding result in degenerate progeny, malformations and sterility occur frequently in these species because of its allogamous mode of reproduction. Therefore, the establishment of inbred lines by conventional selfing methods was found to be difficult (Cappadocia and Vieth, 1990). Developments in tissue culture and methods of molecular genetics now give an alterative approach to change single characteristics in these genotypes. Furthermore, possibilities of producing completely new characteristics are no longer limited by the natural genetic variation existing in the target species, since genes of any origin can be transferred into the pre-existing gene pool. In previous work, adventitious gerbera shoots were regenerated primarily from flower buds of greenhouse-grown plants (Pierik et al., 1973; Pierik et al.,1975 and Laliberte et al., 1985). However, the regeneration of shoots from leaf blades was successfully obtained by Hedtrich (1985). On the other hand, Jerzy and Lubomski (1991) were the first to demonstrate effective regeneration of adventitious shoots from *in vitro* explants of 28 gerbera cultivars. In this work shoots were regenerated directly from petioles or from obtained calli at the base of petioles. Similarly, Orlikowska et al. (1999) regenerated gerbera from callus produced from petioles of the youngest 3 - 4 leaves detached from auxillary shoots produced *in vitro*. In addition, bud regeneration was obtained from a clone of *Gerbera hybrida* Bol. L. leaf explants by Reynoired et al. (1993). Kumar et al. (2004) were able to regenerate adventitious shoots from leaf and petiole pieces of *Gerbera jamesonii*. They reported that about 75 - 77 % of the calli from both types of the explants which produced 12 - 15 shoots/callus.

Agrobacterium-mediated gene transfer method have been established before for a commercial gerbera variety, but still, to date, general transformation protocols suitable for a range of elite varieties have not been developed. Elooma et al. (1993) used *Agrobacterium tumefaciens*-mediated transformation to introduce an antisense gene for chalcone synthase into *cv.* Terra Regina. Whereas, Nowak et al. (1997) introduced the *gus* and *npt* II marker genes into five gerbera cultivars using *Agrobacterium* mediated transformation. In the present investigation, an attempt has been performed to develop regeneration system in *Gerbera jamesonii* using the juvenile leaves as explants in order to improve the breeding work to be more efficient and rapid for producing gerbera plants which have novellas desired traits.

2 Materials and methods

2.1 Cultures condition

In vitro gerbera (*Gerbera jamesonii*) var. SHTC3 plantlets were kindly obtained from SHTC Company, (Egypt). All regeneration and transformation treatments were carried out using the MS medium (inorganic salt MS medium, Murashige and Skoog, 1962), containing 30 g/l sucrose and solidified with 2.8 g/l phytagel. The pH was adjusted to 5.8 before autoclaving. Plant materials were maintained at 28 °C ±2 for 16 h light cycle.

2.2 Explant preparation

The juvenile leaves with their petioles from the *in vitro* propagated plantlets were used as a source of explants. These leaves were excised at early stage of differentiation and the axillary buds were removed at their proximal ends then incubated in culture room for seven to ten days until producing the juvenile leaves.

2.3 Callus induction stage

Explants were evaluated for their ability to produce callus by culturing on 53 MS-based media containing different concentration and combination of plant growth regulators (Table 1). Each treatment consisted of 100 explants derived from juvenile leaves with 10 explants/plat and the experiment was repeated twice. Cultures were incubated for 3 - 4 weeks and the reported data are mean of the two experiments.

2.4 Shoot formation

In order to evaluate the shoot formation ability, the produced calli were cultured on two different shoot formation media (2B and 4B), both contain 2 mg/l NAA but with different concentration of BAP, one has 2.0 mg/l (2B) and the other has 4 mg/l (4B). The calli which did not produce

Table 1:
Composition of callus induction media

Medium	Growth hormones (mg/l)		Medium	Growth hormones (mg/l)		
	BAP	NAA		BAP	ABA	
Ge1	1.0	0	Ge32	0.5	0.1	
Ge2	1.5	0	Ge33	1	0.4	
Ge3	2.0	0	Ge34	1.5	0.5	
Ge4	3.0	0	Ge35	1.0	0.5	
Ge5	1.0	0.5	Ge36	2.0	0.25	
Ge6	1.5	0.5	Ge37	3.0	0.25	
Ge7	2.0	0.5	Ge38	1.0	0.25	
Ge8	3.0	0.5	Ge39	2.0	0.5	
Ge9	1.0	1.0	Ge40	3.0	0.5	
Ge10	1.5	1.0		BAP	ZEA	IAA
Ge11	2.0	1.0	Ge41	0.1	0.1	0.02
Ge12	3.0	1.0	Ge42	0.2	0.2	0.2
Ge13	1.0	2.0	Ge43	0.5	0.5	0.3
Ge14	1.5	2.0	Ge44	1.0	1.0	0.1
Ge15	2.0	2.0	Ge45	1.5	1.5	0.2
Ge16	3.0	2.0	Ge46	2	3	0.4
Ge17	0.2	0.15	Ge47	2.5	3.5	0.5
Ge18	0.5	0.2	Ge48	3	2	0.4
Ge19	1.0	0.5	Ge49	3.5	2.5	0.4
	Kin	NAA		BAP	NAA	IAA
Ge20	1.0	0	Ge50	2		0.1
Ge21	2.0	0	Ge51	2	0.1	0.1
Ge22	3.0	0	Ge52	2		0.1
Ge23	1.0	0.5	Ge53	2.5	0.45	
Ge24	2.0	0.5	Ge54	5.0		0.05
Ge25	3.0	0.5	Ge(A)	2.00		1.00
Ge26	1.0	1.0	Ge(M)	2.0	0.1	0.1
Ge27	2.0	1.0	Ge(P)	2.5	0.45	
Ge28	3.0	1.0	Ge (T)	5.0		0.05
Ge29	1.0	2.0				
Ge30	2.0	2.0				
Ge31	3.0	2.0				

shoots after 3 - 4 weeks were re-cultured on fresh shoot formation media.

2.5 Root formation and adaptation

The produced shoots were transferred to the rooting medium containing 40 µg/l NAA Hussein (2003) and incubated for 3 - 4 weeks. Subsequently, rooted plantlets were acclimatized in pots containing soil composed of peat-moose: sand: clay (1:1:1, v:v:v), covered with plastic bags to increase the humidity and grown under a photoperiod of 16/8 h (light/dark) in controlled greenhouse conditions. Plants were hardened by removing the plastic bags after 7 - 10 days.

3 Results

A regeneration system for gerbera was established using the juvenile leaf explants and MS media with different hormone combinations for producing callus. It was observed from the obtained results that not all media could promote the callus formation, in addition, callus formation percentage varied from 0 to 95 among the media. It was recorded that only five media (Ge36, GeA, GeM, GeP and GeT) showed high percentage of callus formation ranged between 90 - 95 %. After few days of culturing the explants enlarged and most of calli were formed on the petiole bases. It is also obvious that few shoots regenerated

from the callus during incubation on the callus induction media (data not shown). Produced calli enlarged and became yellow-brown nodular semi compact calli (Figure 1). Subsequently, the organogenic calli produced on different media were transferred to two different shoot formation media to evaluate their ability to shoot development. The unresponded calli were transferred to a fresh medium for another 3 - 4 weeks. The regeneration efficiency was calculated during the first and the second 4 weeks and they varied among the treatments. Generally, medium 2B containing 2 mg/l BA and 2 mgl IAA revealed higher shoot development percentage (ranged between 6.6 to 36.6) than the medium 4B which containing 4 mg/l BAP and 2 mg/l IAA (ranged between 3.0 to 28.3), expect in the case of using callus produced from medium GeP which gave 13.30 % on the first period and 15 % on the second. However the highest regeneration efficiency (36.6) was obtained on media Ge36/2B. Shoots were performed during 3 - 8 weeks as it gave a percentage of 16.6 responded calli at the first incubation period and a percentage of 20 at the second incubation period as shown in Table 2. Figure 2 illustrate the shoot regeneration from callus on shoot formation medium. The number of produced shoots/callus was taken in consideration, data in Table 2 show that the average numbers of shoots/callus were higher during the first 4 weeks than that produce during the second, expet in that case of the GeP calli. The mean number of shoots regenerated per callus ranged from 1.00 to 2.60 during the first period while it ranged between 1 to 2.5 shoots/callus during the seconed. The highest number of shoots/callus was obtained with media Ge36/2B, however, the lowest was on media GeP/2B.

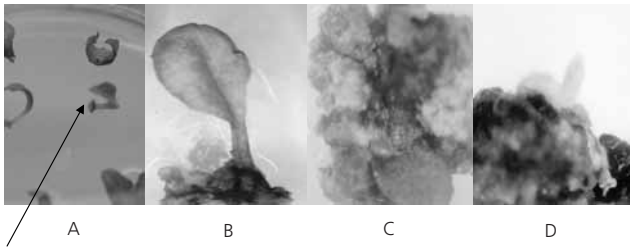

Figure 1:

Callus formed from the juvenile leaf explants of gerbera
A: Juvenile leaf explants. B: Callus produced at the end of petioles of about 2 weeks. C: Produced callus after 3 weeks. D: Bud initiation from callus during maintaining on the callus induction medium.

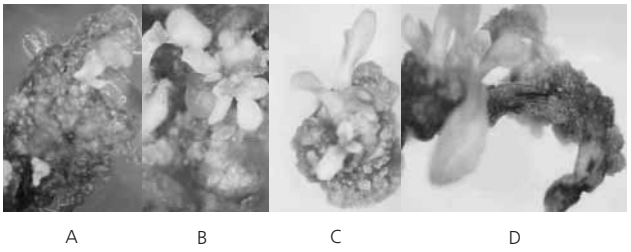

Figure 2:

Developed gerbera shoot obtained from the compact callus during 3 - 4 weeks of subculturing on shoot differentiation medium

Developed shoots were successfully rooted when culturing on medium composed of MS supplemented with 40 µg/l NAA. Roots started appearing after seven to ten

Table 2:

Shoot formation rate of gerbera calli obtained on different induction media

Media		***First subculture***		***Second subculture***		**Total % of responded callus of 2 months**
		% of responded callus after one month	**Average number of shoots/callus**	**% of responded callus after two months**	**Average number of shoost/callus**	
Ge36	2B	16.6	2.60	20.0	1.25	36.6
	4B	3.00	1.00	10.0	1.00	13.00
GeA	2B	26.60	1.30	0.00	0.00	26.60
	4B	10.0	2.50	0.00	0.00	10.00
GeM	2B	6.60	1.00	0.00	0.00	6.60
	4B	3.00	2.00	15.0	1.50	18.0
GeP	2B	10.0	1.00	0.00	0.00	10.0
	4B	13.30	1.25	15.0	2.50	28.30
GeT	2B	20.0	1.50	0.00	0.00	20.0
	4B	6.60	1.50	25.0	1.20	2.50

days of subculturing. All of the transferred shoots produced roots during the 3 - 4 weeks (Figure 3). Rooted plants were then acclimatized in the greenhouse as described before (Figure 4).

A B

Figure 3:
Rooted gerbera plantlets on the rooting medium after 3 weeks

A B

Figure 4:
Gerbera plants under acclimatization stage

4 Discussion

The establishment of a transformation method is an integrated process that involves many different factors, such as the choice of explant, culture conditions, and transformation (Dessoky et al., 2006). Various factors involved in the regeneration have been considered, including developmental stage of the leaves, growth regulators in the culture medium and genotype dependent (Reynoird et al., 1993 and Orlikowska et al.,1999). We established a long term regeneration system of gerbera (*Gerbera jamesonii*) using the juvenile leaf as an explant. Previous work for gerbera regeneration showed that petioles of the youngest 3 - 4 leaves detached from auxillary shoots produced *in vitro* were successfully used as explants (Reynoired et al., 1993; Jerzy and Lubomski, 1991; Orlikowska and Nowak, 1997; Orlikowska et al.,1999 and Kumar et al., 2004). In current study, a protocol including one month of callus induction stage and 3 - 8 weeks of shoot formation stage with two phases has been applied on gerbera (*Gerbera jamesonii*) var. SHTC3. This protocol gave a rate of shoot regeneration efficiency of 36.6 %. The use of long-term regeneration protocol for gerbera is requested (Orlikowska et al.,1999) for successful transformation, as it is important that regeneration is maintained over a relatively long period in which organic calli is first produced from single transformed cells, then shoots are regenerated from calli. In addition, Orlikowska and Nowak, (1997) reported that directly regenerated shoots were either not transformed or not stably transformed. In current study, the maximum callus induction and growth were on all media containing BAP at different concentration. Aswath and Choudhary (2002) and Kumar et al. (2004) reported that BAP produced compact, nodular callus from gerbera leaves or petioles. Our results refer that the range of the main number of regenerated shoot/callus is ranged between 1.0 and 2.6 which depend on the media as well as the phase of shoot formation stage. The obtained number of shoots/callus is near to the results obtained previously with Orlikowska et al. (1999), they stated that it dependent on genotype and recorded that it ranged from 1.9 to 2.5 shoots in the first passage but it increased from 4.4 - 7.3 shoots in the fourth passage. However, Kumar et al. (2004) obtained high numbers of regenerated shoots/explants as it reached upto 11 shoots/explant. Results obtained in this work showed that the roots were successfully developed on 40 g/l NAA. Presence of NAA was also used but in combination with BAP for root formation by Kumar et al. (2004). Aswath and Choudhary (2002) successfully rooted the gerbera shoots on NAA, IAA or free hormone medium.

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