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Effect of Cultivar and Nutrient Medium on *In Vitro* Shoot Formation on Capitulum Explants of Gerbera (*Gerbera jamesonii* Bolus)

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Abstract: The objective of this study was to examine the shoot formation capacity of the capitulum explants of some gerbera cultivars in different nutrient media. Immature capitulum explants of the gerbera cultivars Ameretto, Red Bull, Ruby Red and Yanara were cultured on Pierik medium (P) with different concentrations of benzyladenine (BA) and Radice and Marconi medium (RM). The main P and RM media were used either in liquid form using cotton wool as support or solidified with agar. There was a significant interaction between the different media and the gerbera cultivars in all the parameters measured. The gerbera cultivars showed different responses to various media compositions with regard to shoot formation on the capitulum explant.There was a general increase in vitrification rate with the increase in the BA concentration in the medium. Shoots formed in liquid media showed a high vitrification rate.

Key words: Benzyladenine; capitulum; *Gerbera jamesonii*; liquid medium; vitrification

INTRODUCTION

Gerbera is one of the most popular ornamental flowers in the world with more than 200 varieties. It is the fifth most important cut flower in the world after *rose, carnation, chrysanthemum*, and *tulip.* Gerbera is cultivated in more than 25 countries of the world on a total of about 1200 hectares exclusively in greenhouses. Of this cultivated area almost 50% is found in Holland and Italy. Germany is one of the biggest markets (importer) for the gerbera flowers. Next comes France, Great Britain,

Eastern Europe, Japan, U.S.A., Italy and Holland (Mercurio 2002). Gerbera can be used as a cut flower, a potted flowering plant and a bedding plant. Gerbera can be propagated sexually by seeds. However seed propagation is faced by the problem that the cultivated gerberas are extremely heterozygous and their seedlings are not uniform (Murashige *et al*. 1974). Consequently, a producer must grow thousands of seedlings to harvest 100 flowers of the same color and form at once (Rogers and Tjia 1990). Gerbera can also be propagated vegetatively by the division of the rhizome, but this method is much too slow to be commercially practical (Murashige *et al.* 1974). Since the 1970s, tissue culture has become the predominant method for commercial propagation of gerberas. This system results in uniform, free-branching, vigorous, pathogen-free plantlets available at any time for greenhouse planting (Rogers and Tjia 1990). By clonal multiplication through tissue culture a large number of plants can be produced in a short time. A million fold increase in gerbera plants is possible in one year's time starting with one plant (Murashige *et al*. 1974).

Pierik *et al.* (1975) obtained shoots from fully developed gerbera capitulum explants using a medium with 10 mg/l BA. Pawlowska (1979) and Mohamed and Özzambak (2007) studied the capacity of shoot formation of 3 stages of gerbera capitulum: fully developed capitulum (stage a), when the flower stem reaches 15-20 cm in length and before the appearance of the ray florets (stage b) and immature capitula of 1-1.5 cm in diameter (stage c). Stage c was found most suitable for shoot induction. Laliberte *et al.* (1985) reported that immature capitula 0.5–0.7cm in diameter were up to 10 times as productive as fully developed ones. They also reported that 'Mardi Grass' capitulum explants responded best to the MS medium with 1.0 mg/l BA and 0.1 mg/l IAA. However, the vigour and number of shoots decreased when the concentration of BA was raised to 2.0-3.0 mg/l. Radice and Marconi (1998) obtained shoots from fragments of young gerbera capitulum (diameter =1cm) explants using a medium with 2 mg/l BA.

The objective of this study was to examines the shoot formation capacity of the capitulum explants of some gerbera cultivars by testing various media compositions.

MATERIALS AND METHODS

This research was carried out at the tissue culture laboratory of the Department of Horticulture – Faculty of Agriculture – University of Ege – Turkey. Immature capitula of the gerbera cultivars Ameretto, Red Bull, Ruby Red and Yanara were used as explants. Explants were firstly washed intensively with tap-water then soaked in a 70% (v/v) ethanol for a few seconds then immersed for 90 minutes in a 15% (v/v) clorox (1.5% sodium hypochlorite) to which 1-2 drops of tween 80 were added. Explants were then rinsed three times with sterile distilled water. Sterilization procedures were carried out in sterile glass jars. After sterilization each capitulum was divided into 4 segments and placed horizontally onto the medium. Two main media were used which were :-
(i) Pierik *et al*. (1975) medium **(P)**: ½ MS (Murashige and Skoog, 1962) macroelements + Heller's (1953) microelements + Na Fe EDTA 25 mg/l + Thiamine HCl 1.0 mg/l + Benzyladenine (BA) 10 mg/l + IAA (Indole-3-acetic acid) 0.1 mg/l + Sucrose 10 g/l**.** (ii) Radice and Marconi (1998) medium **(RM)** : ½ MS elements + Adenine sulphate 80 mg/l + BA 2 mg/l + IAA 0.1 mg/l + Sucrose 10 g/l.

The following modified **P** and **RM** media were compared:

- P1 : P + agar 7g / l + BA 2 mg/l
- P2 : P + agar 7g / l + BA 5 mg/l
- P3 : P + agar 7g / l + BA 7 mg/l
- P4a : P + agar 7g / l + BA 10 mg/l
- P4c : P + BA 10 mg/l + cotton wool as a support (liquid medium)
- RMa : RM + agar 7g / l + BA 2 mg/l
- RMc : RM + cotton wool as a support (liquid medium) + BA 2 mg/l

Culture media were prepared from stock solutions of the macroelements, microelements, vitamins and growth regulators. Sucrose and agar were added to the media during preparation. Cotton as a support was put at the bottom of the glass jars before pouring media into them. The pH of all media was adjusted to 5.6 using 1 N HCl or 1 N NaOH prior to autoclaving. Media were poured into jam glass jars and sterilized in the autoclave at 15 psi and 121°C for 20 minutes. The cultures were first

placed in darkness at 25±1°C for 2 weeks and subsequently placed in continuous florescent light (26.0 μmol m^{-2} s^{-1}) at 23±1°C for another 7 weeks. The treatment combinations (gerbera cultivar x medium) were arranged in a completely randomized design as a factorial (a 2-factor) experiment with 4 replications. Three capitulum segments were placed per glass jar. Statistical analysis was done using the SAS program (SAS Version 6.12, SAS Institute Inc., Cary, NC). Differences between means were assessed with the Duncan's Multiple Range Test at $P \leq 0.05$. The parameters measured were number of capitulum explants that formed shoots, number of shoots per explant, shoot length in mm., time (weeks) from placing explants onto the medium till shoot formation and vitrification rate (0–5 score) which was determined visually by observing vitrification symptoms where lower score values indicate lower vitrification rate .

RESULTS

There was a significant interaction between the different media and the gerbera cultivars in all the parameters measured (Tables 1, 2, 3, 4, 5).

Number of capitulum explants that formed shoots

While the mean number of capitulum explants forming shoots in the gerbera cultivars Red Bull, Ruby Red and Yanara was not affected by the medium (the difference between the media was not significant), the cultivar Ameretto showed significantly the highest number of explants forming shoots in the liquid medium P4c (10 mg/l BA and supported with cotton) followed by the media P4a (10 mg/l BA and solidified with agar) and P3 (7 mg/l BA). In the media with low BA concentrations (P1, P2, Rma, RMc) the cultivar Ameretto showed the lowest number of explants that formed shoots (Table 1).

Number of shoots per explant

The cultivar Ameretto gave approximately the same number of shoots per explant in all the media (the difference between the media was not significant). Red Bull gave significantly higher number of shoots per explant (1.8 shoots) in the media P1, P3 and P4c compared to the media RMa and RMc (1.0 shoot). Ruby Red gave significantly higher number of shoots per explant (2.5 shoots) in the media RMa and RMc compared to the media P2, P3, P4a and P4c (1.0–1.5 shoots). Yanara gave significantly

higher number of shoots per explant (2.0 shoots) in the medium RMc followed by P4c (1.8 shoots). compared to the media P1, P2, P3, P4a and RMa (table 2) .

Shoot length (mm)

The cultivar Ameretto gave significantly the longest shoot (24.3 mm) in the medium RMa compared to all the other media. Red Bull showed significantly longer shoot (27.0 mm) in the media P1 and P4a compared with the media P2, P4c, RMa and RMc (6.0–18.0 mm). Ruby Red gave significantly the longest shoot (34.3 mm) in the medium RMa compared to all the other media.Yanara gave significantly the longest shoot (26.3 mm) in the medium P1 compared to a the media P2, P3, P4a, P4c and RMc (Table 3) .

Time of shoot formation (weeks)

As shown in Table 4, the cultivar Ameretto had a general trend to form shoots earlier in the media with higher BA concentrations. It formed shoots in 5.0 weeks in P4c (liquid medium with 10 mg/l BA) and in 6.5 weeks in both P1 (2.0 mg/l BA) and P2 (5.0 mg/l BA). Red Bull and Yanara had a general tendency twards early shoot formation in media with low BA concentrations. Red Bull showed late shoot formation in the liquid media P4c with 10.0 mg/l BA (5.9 weeks) and RMc with 2 mg/l BA (6.0 weeks). Yanara showed early shoot formation in the same two liquid media (5.1 weeks and 4.4 weeks respectively). Ruby Red formed significantly earlier shoots in RMa and RMc media (4.0 weeks) followed by P3 medium (5.0 weeks). It formed shoots in 6.0 weeks in the medium P1.

Vitrification rate (0 – 5 score)

With respect to the semisolid media P1, P2, P3, P4a and RMa , there was a general increase in vitrification rate (increase in score) with the increase in the BA concentration in the medium (from 2.0–10.0 mg/l) in Ameretto, Red Bull and Yanara. Ruby Red showed a significant difference only between the tow media RMa (2 mg/l BA) and P4a (10.0 mg/l BA) where the vitrification score was 0.0 and 1.8 respectively. All cultivars (except Ruby Red in RMc) showed the highest vitrification (highest score) in the liquid media P4c and RMc. Ruby Red showed the lowest vitrification rate

(1.3) which might idicate that it is rather resistant to vitrification. The highest vitrification rate (3.6 and 3.4 scores) was shown by the liquid media P4c and RMc respectively (Table 5).

Table 1. Effect of different media on the mean number of capitulum explants forming shoots in the gerbera cultivars Ameretto, Red Bull, Ruby Red and Yanara after 9 weeks of culture

Media	Gerbera cultivars				Medium mean
	Ameretto	Red Bull	Ruby Red	Yanara	
P1	1.0c	1.5a	2.0a	1.8a	1.6b
P2	1.5c	2.0a	1.8a	2.0a	1.8ab
P3	3.0b	1.5a	2.0a	1.5a	2.0ab
P4a	3.0b	1.5a	1.8a	1.5a	1.9ab
P4c	4.0a	1.5a	2.0a	2.0a	2.4a
RMa	1.3c	2.0a	2.0a	1.5a	1.7b
RMc	1.3c	1.8a	2.0a	2.0a	1.8ab
Cultivar mean	2.1a	1.7a	1.9a	1.8a	

Means followed by the same letter (s) are not significantly different at $P \leq 0.05$.

- P1 : P + agar 7g / l + BA 2 mg/l
- P2 : P + agar 7g / l + BA 5 mg/l
- P3 : P + agar 7g / l + BA 7 mg/l
- P4a : P + agar 7g / l + BA 10 mg/l
- P4c : P + BA 10 mg/l + cotton as a support (liquid medium)
- RMa : RM + agar 7g / l + BA 2 mg/l
- RMc : RM + cotton as a support (liquid medium) + BA 2 mg/l

Table 2. Effect of different media on the mean number of shoots per capiulum explant in the gerbera cultivars Ameretto, Red Bull, Ruby Red and Yanara after 9 weeks of culture

	gerbera cultivars				Medium mean
Media	Ameretto	Red Bull	Ruby Red	Yanara	
P1	1.3a	1.8a	1.8ab	1.0c	1.4ab
P2	1.5a	1.5ab	1.3b	1.3bc	1.4ab
P3	1.0a	1.8a	1.5b	1.3bc	1.4ab
P4a	1.0a	1.3ab	1.5b	1.3bc	1.3b
P4c	1.4a	1.8a	1.0b	1.8ab	1.5ab
RMa	1.3a	1.0b	2.5a	1.3bc	1.5ab
RMc	1.0a	1.0b	2.5a	2.0a	1.6a
Cultivar mean	1.2 b	1.4b	1.7a	1.4b	

Means followed by the same letter (s) are not significantly different at $P \leq 0.05$.

- P1 : P + agar 7g / l + BA 2 mg/l
- P2 : P + agar 7g / l + BA 5 mg/l
- P3 : P + agar 7g / l + BA 7 mg/l
- P4a : P + agar 7g / l + BA 10 mg/l
- P4c : P + BA 10 mg/l + cotton as a support (liquid medium)
- RMa : RM + agar 7g / l + BA 2 mg/l
- RMc : RM + cotton as a support (liquid medium) + BA 2 mg/l

Table 3. Effect of different media on the mean shoot length (mm) in the gerbera cultivars Ameretto, Red Bull, Ruby Red and Yanara after 9 weeks of culture

Media	gerbera cultivars				Medium mean
	Ameretto	Red Bull	Ruby Red	Yanara	
P1	7.5bc	27.0a	11.0c	26.3a	17.9b
P2	6.8c	18.0b	21.5b	8.0d	13 6cd
P3	12.0bc	20.8ab	17.5bc	11.8cd	15.5bc
P4a	12.5bc	27.5a	11.8c	6.8d	14.6cd
P4c	13.0b	7.8cd	16.3bc	10.0cd	11.8d
RMa	24.3a	14.5bc	34.3a	20.0ab	23.3a
RMc	7.3bc	6.5d	22.5b	16.8bc	13.3cd
Cultivar mean	11.9b	17.4a	19.3a	14.2b	

Means followed by the same letter (s) are not significantly different at $P \leq 0.05$.

- P1 : P + agar 7g / l + BA 2 mg/l
- P2 : P + agar 7g / l + BA 5 mg/l
- P3 : P + agar 7g / l + BA 7 mg/l
- P4a : P + agar 7g / l + BA 10 mg/l
- P4c : P + BA 10 mg/l + cotton as a support (liquid medium)
- RMa : RM + agar 7g / l + BA 2 mg/l
- RMc : RM + cotton as a support (liquid medium) + BA 2 mg/l

Table 4. Effect of different media on the mean time of shoot formation (weeks) in thegerbera cultivars Ameretto, Red Bull, Ruby Red and Yanara after 9 weeks of culture

Media	gerbera cultivars				Medium mean
	Ameretto	Red Bull	Ruby Red	Yanara	
P1	6.5a	4.4c	6.0a	5.0b	5.5ab
P2	6.5a	4.8bc	5.8ab	5.6 abc	5.7a
P3	5.8ab	4.9bc	5.0c	6.6a	5.6ab
P4a	5.8ab	5.3b	5.9ab	5.9ab	5.7a
P4c	5.0b	5.9a	5.3bc	5.1bc	5.3ab
RMa	5.9ab	4.9bc	4.0d	5.4 abc	5.0b
RMc	5.9ab	6.0a	4.0d	4.4c	5.1b
Cultivar mean	5.9a	5.2b	5.1b	5.4b	

Means followed by the same letter (s) are not significantly different at $P \leq 0.05$.

- P1 : P + agar 7g / l + BA 2 mg/l
- P2 : P + agar 7g / l + BA 5 mg/l
- P3 : P + agar 7g / l + BA 7 mg/l
- P4a : P + agar 7g / l + BA 10 mg/l
- P4c : P + BA 10 mg/l + cotton as a support (liquid medium)
- RMa : RM + agar 7g / l + BA 2 mg/l
- RMc : RM + cotton as a support (liquid medium) + BA 2 mg/l

Table 5. Effect of different media on mean shoot vitrification rate (0–5 score) in the gerbera cultivars Ameretto, Red Bull, Ruby Red and Yanara after 9 weeks of culture

Media	gerbera cultivars				Medium mean
	Ameretto	Red Bull	Ruby Red	Yanara	
P1	0.0d	0.0d	0.8bc	0.0e	0.2d
P2	0.5cd	0.8c	1.0bc	2.8c	1.3c
P3	1.6bc	1.9b	1.0bc	2.8c	1.8b
P4a	1.5bcd	1.1c	1.8b	3.9b	2.1b
P4c	3.0b	3.4a	3.3a	4.8a	3.6a
RMa	0.0d	1.3bc	0.0c	1.0d	0.6d
RMc	4.9a	3.9a	1.0bc	3.8b	3.4a
Cultivar mean	1.6bc	1.8b	1.3c	2.7a	

Means followed by the same letter (s) are not significantly different at $P \leq 0.05$.

- P1 : P + agar 7g / l + BA 2 mg/l
- P2 : P + agar 7g / l + BA 5 mg/l
- P3 : P + agar 7g / l + BA 7 mg/l
- P4a : P + agar 7g / l + BA 10 mg/l
- P4c : P + BA 10 mg/l + cotton as a support (liquid medium)
- RMa : RM + agar 7g / l + BA 2 mg/l
- RMc : RM + cotton as a support (liquid medium) + BA 2 mg/l

DISCUSSION

The gerbera cultivars showed different responses to various BA concentrations. This result is in conformity with that of Van Son (2007) who worked with the gerbera varieties Arianna, Bonnie and Tobia. The variety Arianna showed higher number of explants forming shoots and higher number of shoots per explant with lower concentration of BA (3 mg/l), whereas variety Bonnie performed well at medium concentration of BA (5 mg/l). Variety Tobia showed higher number of explants responded, number of shoots per explant with higher concentration of BA (10 mg/l).
In this study the observed differences between the gerbera cultivars in terms of number of explants forming shoots, number of shoots per explant, shoot length, time of shoot formation and vitrification rate might be attributed to genotype differences. Murashige *et al.* (1974) used the shoot tip for clonal multiplication of different gerbera cultivars.They observed differences among cultivars with respect to ease of culturability and rates of multiplication *in vitro*. Chu and Huang (1983) succeeded in producing shoots from the gerbera flower stem (scape) of the gerbera cultivars Arendsoog and Super Giant. The scape explants of the cultivar Beatrix formed more callus. There was no shoot and little callus formation on the scape explants of the cultivar Continent.

This difference noticed among genotypes could be attributed to interaction effect of endogenous and exogenous growth regulators. Also, every genotype has a specific range of optimum growth regulator concentration (Deepaja 1999). Pierik *et al.* (1979) reported that success of gerbera propagation using the capitulum explant depends on the individual cultivar and the concentration of the exogenous cytokinins. With capitulum explants, shoot formation was found very low for some gerbera cultivars regardless of the level of BA; whereas other cultivars had individual optimum BA levels (Pierik *et al.* 1982).

Other research workers found differences between genotypes in different crops with respect to regeneration capacity *in vitro* (*Rhododendron*: Pierik and Steegmans 1975; *Brassica* spp. Tang *et al.* 2003; *Primula vulgaris*: Schween and Schwenkel 2003; sunflower: Berrios *et al.* 2000). In plant tissue culture, it is now well known that no two genotypes give similar response under a given set of culture conditions (Nehra *et al.* 1989). There

is a wide range of regenerative capacity in the plant kingdom. There are very great differences in cell division and regenerative capacity between plants within a single species (Pierik 1987). Berrios *et al.* (2000) reported that shoot production in a particular explant genotype depends on the culture conditions, and principally on the interaction between genotype and medium.

Ameretto showed significantly the highest number of explants forming shoots in the liquid medium P4c (10 mg/l BA and supported with cotton wool) compared to the other media (Table 1). Yanara gave the highest number of shoots per explant (2.0 shoots) in the liquid medium RMc followed by the liquid medium P4c (1.8 shoots) compared to the media P1, P2, P3, P4a and RMa (Table 2). As shown in table 4, Red Bull showed late shoot formation in the liquid media P4c with 10.0 mg/l BA (5.9 weeks) and RMc with 2 mg/l BA (6.0 weeks). Yanara showed early shoot formation in the same two liquid media (5.1 weeks and 4.4 weeks respectively). While some plants do not grow well in liquid medium, others grow well in it. Growth and development in a liquid medium is often very good, since if the plants are submerged they can readily take up nutrients, growth regulators etc. on all sides , in contrast to growth on agar where there is only basal contact. Growth on liquid medium also means that any exudates from the explants is diluted more readily than on agar where local accumulation may occur **(**Pierik 1987). Shoot production of *Rhododendron* was found to be 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 in terms of fresh weight, dry weight and number of shoots produced between the supports used. Best response was obtained with liquid agitated medium. The use of liquid medium in tissue culture is often described as a means of reducing the cost of micropropagation (Alvard *et al*. 1993). 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.

In this experiment the increase in vitrification rate might be attributed to the use of liquid media and the increase in cytokinin (BA) concentration. In studies on *in vitro* propagation of Carnation (*Diathus caryophyllus* L.), Manreet *et al*. (1998) and Kharrazi *et al.* (2011) found that high cytokinin (BAP) level had caused vitrification of shoots. Vitrification is an undesirable physiological disorder of *in vitro* tissues where leaves and sometimes stems show glassy, transparent, succulent or wet and often swollen appearance (Chawla 2002). It is a major problem in the tissue culture industry since it can affect shoot multiplication and culture vigour (Hammerschlag 1986) and can impede the successful transfer of micropropagated plants to *in vivo* conditions. Up to 60% of affected plants fail to acclimatise (Paques and Boxus 1987), thereby limiting the application of *in vitro* techniques for mass propagation. Vitrification is especially common if the plant has too much water available, this being the case in liquid media, or if the medium has a low agar concentration. It is associated with high cytokinin levels (Pierik 1987).

Differences between the gerbera cultivars in terms of number of explants forming shoots, number of shoots per explant, shoot length, time of shoot formation and vitrification rate might also be attributed to the interaction between the gerbera genotypes and the different media.
For plantlet production the resultant shoots were multiplied, rooted and acclimatized according to our previous procedure (Mohamed and Özzambak 2007).

We can conclude that several factors (gerbera cultivar, BA concentration, the physical state of the medium and the interaction between the gerbera genotypes and the different media) can influence shoot regeneration and vitrification in gerbera. The gerbera cultivars showed different responses to various media compositions with regard to shoot formation on the capitulum explant and vitrification.There was a general increase in vitrification rate with the increase in the BA concentration in the medium. Shoots formed in liquid media showed a high vitrification rate. The study suggests the use of agar solidified medium with lower concentration of BA for obtaining the desirable shoot regeneration with less vitrification rate.

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