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Effect of Cultivar and Gelling Agent on *In Vitro* Multiplication of Gerbera (*Gerbera jamesonii* Bolus) Shoots

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Abstract: An experiment was conducted to study the effect of cultivar and concentration of agar and gelrite on *in vitro* multiplication of gerbera (*G.jamesonii*) shoots. Sterile *in vitro* growing shoots of the gerbera cultivars Ameretto, Red Bull, Ruby Red, Yellowish green and Crillo were placed in glass jars containing nutrient media with different concentrations of agar and gelrite. There was a significant interaction between the gelling agents' concentrations and the gerbera cultivars. There was a decrease in the multiplication and vitrification rates with the increase of the concentration of the different gelling agents in the nutrient medium.

Key words: *Gerbera jamesonii* ; gelling agent ; shoot multiplication

INTRODUCTION

Gerbera (*Gerbera jamesonii* Bolus), commonly known as Transvaal Daisy, Barberton Daisy or African Daisy, is an important commercial flower grown throughout the world in a wide range of climatic conditions. It ranks fifth in the international cut flower market, and is ideal for beds, borders, pots and rock gardens. The flowers are of various colours that suit very well in different floral arrangements. The cut blooms also have a long vase life of

about 7 to 8 days. *G. jamesonii* is generally propagated by division of suckers or clumps (rhizomes). Propagation through seed is not preferred as the plants exhibit heterozygosity and non-uniformity. Also, the improved semi-double and double cultivars do not set seeds. Propagation by division of suckers or clumps gives true to type plants, but the multiplication rate is very low. Many new varieties are being introduced every year. To popularize these varieties and also to meet the demand for quality planting material of elite varieties, there is a need to develop technology for rapid multiplication. This could be accomplished through micropropagation technique.

The choice of gelling agent is very important for *in vitro* plant regeneration (Debergh 1983; Singhas 1984; Pochet *et al*.1991). Agar is the most popular solidifying agent. *In vitro* growth may be adversely affected by increasing agar concentration.With higher concentrations , the medium becomes hard and does not allow the diffusion of nutrients into the tissue. Gelrite at 0.2 % is one of the alternatives to agar (Chawla 2002). Agar was thought to be biologically inert but a number of reports on its adverse effects have been published (Romberger and Tabor 1971; Debergh *et al*. 1981; Debergh 1983), including batch-to-batch variability, inhibition of growth and presence of impurities. It is generally accepted that inorganic compounds in gelling agents and the dynamics of the interaction of gelling agent-medium-tissue play a major role during *in vitro* tissue growth (Scholten and Pierik 1998). In some studies different types of agar were found to have no significant effect on the performance of the explant of *Nicotiana tabacum* (Puchooa *et al*.1999) and Lactuca sp. (Roberts *et al*.1984). In other studies, different types of gelling agents were found to have significant effect on the performance of the explants of *Helianthus annuus* (Berrios *et al*. 1999), *Lactuca* sp. (Roberts *et al*. 1984) and *Rosa damascena* (Kumar *et al*. 2003).

Vitrification (Hyperhydration/ hyperhydricity) 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 (Pâques and Boxus 1987), thereby limiting the application of *in vitro* techniques for mass propagation. It was mentioned in many reports that vitrification is a result of Low agar concentration (Debergh 1983; Ziv *et al*. 1983; Von Arnold and Eriksson 1984; Leonhardt and Kandeler 1987; Pierik 1987).

The objective of this experiment was to study the effect of concentration of two types of agar and gelrite on *in vitro* shoot multiplication of the gerbera cultivars Ameretto, Red Bull, Ruby Red, Yellowish green and Crillo.

MATERIALS AND METHODS

This study was carried out at the tissue culture laboratory of the Department of Horticulture, Faculty of Agriculture, University of Ege – Turkey.

Plant material

Sterile *in vitro* growing shoots of the gerbera cultivars Ameretto, Red Bull, Ruby Red, Yellowish green and Crillo were used as explants.

Culture Medium

Huang and Chu (1985) medium (**HC**) was used: ½ MS (Murashige and Skoog 1962) inorganic nutrients + MS vitamins and amino acids + BA (Benzyladenine) 5 mg/l + IAA (Indole-3-acetic acid) 0.1 mg/l + Sucrose 20 g/l.

HC medium was solidified with the following gelling agents : -

Agar 1 (a_1) : Bacteriological agar (agar No.1) for *in vitro* diagnostic use. Unipath LTD. England.

Agar 2 (a_2) : Agar – agar ultrapure (granulated) for microbiology. Merck – Germany.

Gelrite (G) .

HC medium was solidified with the following concentrations of these gelling agents : -

HC + a_1 7 g/l
HC + a_1 8 g/l
HC + a_1 9 g/l
HC + a_2 3 g/l
HC + a_2 5.5 g/l
HC + a_2 8 g/l
HC + G 1.5 g/l
HC + G 2 g/l

The pH of all media was adjusted to 5.6 using 1 N HCl or 1 N NaOH prior to autoclaving. Twenty-five ml of medium were poured into 100 ml jam glass jars and sterilized in the autoclave at 1.05 kg cm^{-2} and 121°C for 20 minutes. The cultures were placed in continuous florescent light (26.0 µmol m^{-2} s^{-1}) at 23±1°C. The treatment combinations (gerbera cultivar x medium) were arranged in a completely randomized design as a factorial (a 2-factor) experiment with 4 replications. Two shoots were placed per glass jar. The parameters measured were multiplication rate (mean numbr of shoots/ explant), mean shoot length (mm) and mean vitrification rate (0 – 5 score) which was determined visually by observing vitrification symptoms where lower score values indicate lower vitrification rate.

Statistical analysis was done using the SAS program (SAS Version 6.12, SAS Institute Inc., Cary, NC). Means were separated for significant using Duncan's Multiple Range Test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Multiplication rate (numbr of shoots / explant):
There was a significant interaction between the different media and the gerbera cultivars (Table 1). While the cultivars Ameretto , Red Bull , Ruby Red and Crillo showed some differences in the multiplication rate among the different media , the cultivar Yellowish green gave approximately the same multiplication rate in all media. Ameretto showed a significant decrease in multiplication rate as a_1 increased from 8.0 g/l to 9.0 g/l. Ameretto showed also a decrease in multiplication rate as a_2 increased from 5.5 g/l to 8.0 g/l

but the decrease was not significant (Table 1). Red Bull showed a significant decrease in multiplication rate as a_1 increased from 8.0 g/l to 9.0 g/l . Red Bull showed also a significant decrease in multiplication rate as a_2 increased from 3.0 g/l to 8.0 g/l (Table 1).
Ruby Red and Crillo also showed a decrease in multiplication rate with the increase of the concentrations of both a_1 and a_2 but the decrease was not significant.
Ameretto showed significantly higher multiplication rate in a_1 at 7.0 and 8.0 g/l than a_2 at 3.0 and 5.5 g/l. Red Bull showed significantly higher multiplication rate in a_1 at 8.0 g/l than a_2 at 5.5 g/l. Ruby Red , Yellowish green and Crillo showed no significant difference in multiplication rate between a_1 and a_2 (Table 1).

Moreover, Ameretto showed significantly higher multiplication rate in G at 1.5 g/l than a_1 at 9.0 g/l and a_2 at 8.0 g/l . It also showed significantly higher multiplication rate in a_1 at 7.0 and 8.0 g/l than G at 2.0 g/l. Ruby Red showed significantly higher multiplication rate in a_2 at 3.0 and 5.5 g/l than G at 2.0 g/l. The cultivars Red Bull , Yellowish green and Crillo showed no significant difference in multiplication rate between agar and gelrite (Table 1).

The decrease in multiplication rate with the increase of the concentrations of agar mentioned above could be explained by the fact that high agar concentration affected the physical state of the medium which became hard and did not allow the diffusion of plant growth regulators and nutrients into the plant tissue (Bornman and Vogelman 1984 ; Chawla 2002). Karim *et.al*.(2003) cultured nodal explants of chrysanthemum on MS medium having various concentrations of agar and found that percentage of explants showing shoot proliferation, number of shoots per explants and shoot length were lowest at the highest agar concentration. In artichoke micropropagation, Debergh and Ark (Cited by Yalçin 1992) found that by increasing the agar concentration in the medium from 0.6 % to 1.1 % , the multiplication rate decreased from 3.5 % to 1.0 %. Possible explanations for the differences between the gelling agents (a_1, a_2 and gelrite) in shoot multiplication rate include: limited diffusion of medium components and water into the explant

tissue (Stolz 1971), impurities (inorganic elements) found in the gelling agents (Nairn *et al*. 1995) and differences in gel strength (Debergh 1983). Variable concentrations of inorganic salts, as impurities in the agar products could have a strong influence on regeneration ability (Beruto *et al*. 1999).

Table 1**.** Effect of HC medium gelled with agar 1, agar 2 and gelrite on shoot multiplication rate of gerbera cultivars Ameretto, Red Bull, Ruby Red, Yellowish green and Crillo, after 8 weeks of culture

Medium	Amer.	Red Bull	Ruby Red	Yellowish green	Crillo	Mean
HC+a_1 7 g/l	4.3 a	3.3 a	2.7 ab	3.0 a	2.7 a	3.2 a
HC+a_1 8 g/l	4.0 a	3.3 a	2.7 ab	3.0 a	2.7 a	3.1 a
HC+a_1 9 g/l	2.3 c	2.3 bc	2.0 b	2.3 a	2.0 ab	2.2 cd
HC+a_2 3 g/l	3.0 bc	3.0 ab	3.0 a	2.3 a	2.3 ab	2.7 b
HC+a_2 5.5 g/l	3.0 bc	2.3 bc	3.0 a	2.3 a	2.3 ab	2.6 b
HC+a_2 8 g/l	2.3 c	2.0 c	2.3 ab	2.0 a	2.0 ab	2.1 d
HC+G 1.5 g/l	3.7 ab	2.7 abc	2.3 ab	2.3 a	1.7 b	2.5 bc
HC+G 2 g/l	3.0 bc	2.7 abc	2.0 b	2.7 a	2.0 ab	2.4 bcd
Mean	3.2 a	2.7 b	2.5 bc	2.5 bc	2.2 c	

Means in columns or rows followed by the same letter(s) are not significantly different at P= 0.05, according to Duncan's Multtiple Range Test .

Shoot length (mm)

As shown in Table 2, there was a significant interaction between the different media and the gerbera cultivars. While the cultivars Ruby Red and Crillo showed some differences in shoot length among the different media, the cultivars Ameretto, Red Bull and Yellowish green gave approximately the same shoot length in all media. In case of Ruby Red, the only significant difference in shoot length was between the medium HC+ a_2 3g/l (23.3 mm) and the two media HC+a_1 7 g/l (15.0 mm) and HC+a_1 9 g/l (14.3 mm). In case of Crillo, the only significant difference in shoot length was between the medium HC+G 2 g/l (20.7 mm) and the two media HC+a_1 9 g/l (14.0 mm) and HC+ a_2 3 g/l (15.0 mm). Differences between the gerbera cultivars in shoot length among different media might be attributed to genotype

differences and to the interaction between the gerbera genotypes and the different media.

Differences between genotypes with respect to *in vitro* performance were found by many research workers in different crops *e.g. Gerbera jamesonii* (Van Son 2007 and Mohamed and Özzambak 2007); *Brassica* Spp. (Tang *et al.* 2003); *Primula vulgaris* (Schween and Schwenkel 2003). 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). Berrios *et al.* (1999) reported that shoot production in a particular explant genotype depends on the culture conditions, and principally on the interaction between genotype and medium.

Table 2. Effect of HC medium gelled with agar 1, agar 2 and gelrite on shoot length (mm) of gerbera cultivars Ameretto, Red Bull, Ruby Red, Yellowish green and Crillo, after 8 weeks of culture

Medium	Ameretto	Red Bull	Ruby Red	Yel. green	Crillo	Mean
HC+a_1 7 g/l	19.0 a	22.7 a	15.0 b	17.7 a	17.7 abc	18.4 a
HC+a_1 8 g/l	16.0 a	21.0 a	16.3 ab	14.0 a	17.3 abc	16.9 ab
HC+a_1 9 g/l	16.7 a	16.7 a	14.3 b	15.0 a	14.0 c	15.3 b
HC+a_2 3 g/l	14.3 a	21.0 a	23.3 a	15.0 a	15.0 bc	17.7 ab
HC+a_2 5.5 g/l	15.0 a	17.7 a	17.7 ab	14.3 a	20.0 ab	16.9 ab
HC+a_2 8 g/l	14.0 a	17.7 a	17.7 ab	14.0 a	18.3 abc	16.3 ab
HC+G 1.5 g/l	18.3 a	21.7 a	20.0 ab	12.7 a	16.0 abc	17.7 ab
HC+G 2 g/l	17.7 a	22.3 a	17.7 ab	12.3 a	20.7 a	18.1 a
Mean	16.4 b	20.1 a	17.8 b	14.4 c	17.4 b	

Means in columns or rows followed by the same letter(s) are not significantly different at P=0.05 ,according to Duncan's Multtiple Range Test.

Vitrification rate (0 – 5 score)

There was a decrease in the vitrification rate with the increase of the concentration of agar (a_1 and a_2) and gelrite (Table 3). The medium containing the lowest amount of a_2 (HC+a_2 3 g/l) showed significantly the highest Vitrification rate compared with all the other media. The medium containing the lowest amount of a_1 (HC+a_1 7g/l) and that containig 5.5 g/l of a_2 (HC+a_2 5.5 g/l) occupied the second rank of Vitrification rate. Similar results were obtained by Debergh and Ark (Cited by Yalçin 1992) who found that in artichoke micropropagation, increasing the agar concentration in the medium from 0.6 % to 1.1% , decreased the vitrification percentage from 40 % to 0 %. Supporting this result is also the work of Ziv *et al*.(1983) who found that in carnation shoot apex culture, when agar was increased, promotion of normal leaf production was observed. Vitrification of shoots was also observed in guava cultures grown under low agar concentration (Amin and Jaiswal 1989). In micropropagation of sunflower (*Helianthus annuus*), Abdoli *et al*. (2007) reported that the percentage of explants forming shoots and the percentage of hyperhydric shoots increased with decreasing agar concentration and when the agar concentration had increased (0.4 % to 0.8%), the percentage of hyperhydric shoots was significantly reduced from. It was reported that some commercial agars contain components that can control hyperhydricity, while other gelling agents are not capable of hydric control (Nairn *et al*. 1995). The gelling agent used in the medium can be another factor inducing hyperhydration . For example gelrite was found to induce hyperhydration in apple (Pasqualetto *et al.* 1988) and in *Clianthus formosus* (Taji and Williams 1989). However, to be able to explain fully the performances of the gelling agents used in our experiments, chemical and physical analyses of these agents need to be carried out including biologically active organic compounds, the electrical conductivity, the water content, the gel strength, the diffusion rates of water and salts and pH, amongst others. Further studies are needed to examine the effect of other types of agar and agar substitutes on gerbera shoot multiplication.

For plantlet production the resultant shoots were rooted and acclimatized according to our previous procedure (Mohamed and Özzambak 2007). Since

gelrite is more expensive than agar (Puchooa *et al*. 1999), our results support the use of moderate agar concentration, that allow good multiplication rate with good quality shoot.

Table 3. Effect of HC medium gelled with agar1, agar2 and gelrite on vitrification rate (0 – 5 score) of the gerbera cultivars Ameretto, Red Bull, Ruby Red, Yellowish green and Crillo, after 8 weeks of culture

Medium	Ameretto	Red Bull	Ruby Red	Yel. green	Crillo	Means
HC+a_1 7 g/l	1.5 bc	1.8 a	1.0 bc	2.0 b	2.7 a	**1.8 b**
HC+a_1 8 g/l	0.7 de	0.7 b	0.5 cd	0.5 cd	1.3 bc	**0.7 c**
HC+a_1 9 g/l	0.2 e	0.2 b	0.2 d	0.2 d	0.3 d	**0.2 e**
HC+a_2 3 g/l	3.0 a	2.0 a	2.7 a	3.7 a	3.3 a	**2.9 a**
HC+a_2 5.5 g/l	2.0 b	1.7 a	1.7 b	2.0 b	1.7 b	**1.8 b**
HC+a_2 8 g/l	0.3 de	0.3 b	0.3 cd	0.5 cd	0.3 d	**0.4 de**
HC+G 1.5 g/l	1.0 cd	0.5 b	1.0 bc	1.0 c	0.8 cd	**0.9 c**
HC+G 2 g/l	0.8 cde	0.3 b	0.8 cd	0.5 cd	0.3 d	**0.6 cd**
Mean	**1.2 ab**	**0.9 c**	**1.0 bc**	**1.3 a**	**1.4 a**	

Means in columns or rows followed by the same letter(s) are not significantly different at P = 0.05 , according to Duncan's Multtiple Range Test .

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تأثير الصنف و المادة الدعامية على اكثار سيقان الجيربرا داخل الأنابيب

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المستخلص: أجريت هذه الدراسة لبحث تأثير الصنف و تركيز المادة الدعامية (نوعين من الأجار و جلرايت) على اكثار سيقان الجيربرا داخل الأنابيب. أخذت سيقان معقمة من الأنابيب لأصناف الجيربرا Ameretto ، Red Bull ، Ruby Red Yellowish greenو Crillo و زرعت في قوارير زجاجية تحتوي على أوساط غذائية ذات تراكيز مختلفة من الأجار و جلرايت. أظهرت النتائج تفاعلا معنويا بين تراكيز المواد الدعامية وأصناف الجيربرا. كان هنالك نقص في معدلي الاكثار والتزجج مع زيادة تركيز المواد الدعامية المختلفة في الوسط الغذائي.