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Gelling agents and culture vessels affect *in vitro* multiplication of banana plantlets

Y.A. Kaçar[1,2], B. Biçen[1], İ. Varol[1], Y.Y. Mendi[1,2], S. Serçe[3] and S. Çetiner[4]

[1]Department of Horticulture, Faculty of Agriculture,
Çukurova University, Adana, Turkey
[2]Biotechnology Department, Institute of Basic and Applied Sciences,
University of Cukurova, Adana, Turkey
[3]Department of Horticulture, Faculty of Agriculture,
Mustafa Kemal University, Antakya, Turkey
[4]Faculty of Engineering and Natural Sciences,
University of Sabanci, Orhanli, Tuzla, Istanbul, Turkey

Corresponding author: Y.A. Kaçar
E-mail: ykacar@cu.edu.tr

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ABSTRACT. Agar is the most commonly used gelling agent in media for plant tissue culture. Because of the high price of tissue-culture-grade agar, attempts have been made to identify suitable alternatives. The type of culture vessel and lid also affects the gaseous composition inside the vessel as well as light penetration. In turn, the vessel affects growth parameters, such as shoot elongation, proliferation and fresh weight, as well as hyperhydric degradation processes. We examined the effects of different culture vessels, including commercial glass jars, magenta boxes, and disposable containers, as well as different gelling agents (agar-agar, Agargel, Phytagel, and plant agar) on the micropropagation of Dwarf Cavendish bananas in an effort to find a combination that yields large numbers of high-quality seedlings. The different culture vessels did not significantly affect seedling culture success. The medium significantly affected shoot weight. Phytagel resulted in the highest shoot weight (overall mean = 2.4 g), while agar, Agargel and plant agar resulted in 1.7, 2.2 and 2.2 g, respectively. Disposable container/Phytagel and Magenta/Agargel combinations yielded the

highest shoot weights (2.9 and 3.0 g, respectively). Mean shoot length increased progressively with subculture (four subcultures were made). The highest mean shoot length was obtained with Phytagel and Agargel media (6.4 and 6.3 cm, respectively). Shoot number was significantly affected by medium only at subculture 4. Overall, the highest mean shoot length was obtained with the Magenta/Agargel combination (8.5 cm). Phytagel and plant agar gave higher mean shoot number than agar and Agargel (2.1, 2.1 and 1.7 and 1.9, respectively). The costs of the media and of the culture vessels need to be taken into account for final choice of the banana shoot culture system.

Key words: Dwarf Cavendish; Magenta vessel; Agar-agar; Agargel; Phytagel; Plant agar

INTRODUCTION

Banana (*Musa* spp AAA), an important fruit crop of the Musaceae family, is widely grown in developing countries and is the second largest fruit crop in the world, after citrus (Madhulatha et al., 2004). Although initiated by diploid cultivars, banana production is based primarily on triploid cultivars. Most breeding programs aim at generating tetraploid hybrids obtained from crossing established triploid cultivars with a diploid parent genotype (Creste et al., 2004).

Banana production in Turkey began in the 1930s and spread rapidly through the Mediterranean coastal region, which has a mild winter climate. The trend has not continued in recent years, as growers have experienced problems such as nematodes, viruses and fungal diseases. Some of these problems were associated with cultivar selection. The introduction of Dwarf Cavendish and adoption of new growing techniques have recently made banana production more profitable in Turkey. For example, the production of Dwarf Cavendish under plastic covers and protected cultivation was found to be 53% greater than the open-field production (65.5 *vs* 42.8 tons/ha) (Gübbük and Pekmezc, 2004a). Interestingly, Dwarf Cavendish is not a single genotype but is rather a group of clones with similar horticultural attributes. Gübbük et al. (2004) have recently selected new clones with superior horticultural attributes from Dwarf Cavendish genotypes in Turkey, such as Alanya 5, Anamur 10, and Bozyazı 14. These new genotypes are expected to increase production by 150 tons/year in Turkey.

Banana cultivars are vegetatively propagated. Corms, suckers and sword suckers are used as propagation materials, which usually contain pests such as fungi, nematodes, viruses, and insects. Since diseases are often spread through vegetative propagation, there has been a great deal of effort to create disease-free planting material on a large scale through tissue culture, which also has the advantages of speed and effectiveness. Similar trends can be seen in the last 25 years of fruit propagation, especially in tissue culture of clonally propagated rootstocks of apple, pear and cherry (Dennis Jr., 2003). Successful results have been obtained in these crops; however, the cost of culturing may still be considerable for many species.

Bananas can be micropropagated with high multiplication rates, and the production

of highly quality plants is feasible. Many studies have reported on the clonal propagation of *Musa* spp (Cronauer and Krikorian, 1984; Wong, 1986; Nandwani et al., 2000; Kodym and Zapata-Arias, 2004; Muhammad et al., 2004). Micropropagation has become a routine procedure, but the high costs involved have prevented laboratories with limited resources from benefiting from tissue culture technology (Kodym and Zapata-Arias, 2004).

As an example protocol for banana tissue culture, an efficient system was developed for *in vitro* mass propagation of the commercial cultivar *Musa acuminata* cv. Dwarf Cavendish by the cultivation of shoot apices. Shoot apices with 2-3 pairs of leaf primordia were induced from sliced rhizome explants on MS (Murashige and Skoog, 1962) medium supplemented with 6.0 mg/L BA, 150 mg/L Ads and 3% (w/v) sucrose. Multiple shoots were induced from meristematic domes on the same medium. Addition of 1.0-1.5 mg/L IAA to the culture medium and incubation in continuous light (24 h) increased the yield of multiple shoots. The high multiplication coefficient was stably maintained up to the fifth subculture; thereafter, it declined. Rooting was readily achieved upon transferring the shoots onto half-strength MS medium supplemented with 500 mg/L activated charcoal and 2% (w/v) sucrose. Micropropagated plantlets were hardened in a polyethylene house and successfully established in soil. There was no morphological variation among the micropropagated plants (Rout et al., 2001). Gübbük and Pekmezc (2004b) published a protocol refined for Turkish Dwarf Cavendish clones: supplementations with 2 µM TDZ and 1 µM IAA or 20 µM BAP and 1 µM IAA on MS medium, followed by 5 g/L charcoal at the rooting stage, were the best combinations for the *in vitro* propagation of banana types.

Agar is the most commonly used gelling agent in media for plant tissue culture. Since its introduction more than 100 years ago, it has remained the most frequently used gelling agent in culture media employed for microbes as well as plants. The properties of agar that make it the gelling agent of choice are stability, high clarity and resistance to metabolism during culture (Jain and Babbar, 2002). Because of the high price of tissue culture grade agar, attempts have been made to identify suitable alternatives.

There are many commercial grades of gelling agents. The importance of using pure grade gelling agents in experimental tissue culture work is widely acknowledged; however, their use in commercial micropropagation is not necessary. Lower grade gelling agents may contain various ions, sulfonated polysaccharides and long fatty acids, but their effect on plant growth *in vitro* is unknown (Puchooa et al., 1999).

The lid of culture vessels protects the culture medium from microbial infections and prevents excessive evaporation of water from the culture medium. The type of vessel and lid affects the gaseous composition inside the vessel as well as light penetration. Therefore, the growth of tissues in culture (shoot elongation, proliferation, fresh weight increase, and possibly the hyperhydric degradation processes) is also affected (Islam et al., 2005). This study addressed the effect of different culture vessels such as commercial glass jars, Magenta vessels, and disposable culture containers as well as different gelling agents (agar-agar, Agargel, Phytagel, and plant agar) on the micropropagation of Dwarf Cavendish banana.

We evaluated different systems of banana tissue culture to identify the most efficient one yielding many high-quality seedlings. Agar can be the most expensive component of plant tissue culture media. The cost of commercial micropropagation can be reduced if less expensive alternatives are used. Different culture vessels, such as disposable containers, may reduce labor costs in commercial tissue culture laboratories.

MATERIAL AND METHODS

Plant material

The banana cultivar Dwarf Cavendish was obtained from a commercial banana grower. Strong sterilization procedures were used to control contaminants before explants were used in tissue culture. The plants were washed thoroughly for 30 min under running tap water, followed by removal of leaves and roots. The basal parts of plants, including about 2.5 cm of shoot tip, were excised. The explants were treated with 0.05% (w/v) mercuric chloride with a small amount of Tween 20 for 10 min, followed by three rinses with sterile distilled water, and afterward leaves were removed at a length or width of 0.5 cm. The explants were then rinsed in 70% ethanol, soaked in a 10% (v/v) NaOCl with a small amount of Tween 20 solution for 10 min, followed by three rinses with sterile distilled water. This step was repeated twice. Shoot tips were cut longitudinally into two sections and cultured on initiation medium.

Culture conditions

MS basal salt mixture supplemented with Morel vitamins (Morel and Wetmore, 1951), 2 mg/L BA and 4% sucrose was used as initiation and multiplication media. Agar-agar (Sigma A-1296), Agargel (Sigma A-3301), Phytagel (Sigma P-8169), or plant agar (Duchefa P 1001) were added at 7, 5, 3, and 7 g/L, respectively, as gelling agents.

All media were adjusted to pH 5.7 with 0.1 N NaOH or HCl and were autoclaved at 1.05 kg/cm^2 and 121°C for 15 min. Different culture vessels such as Magenta vessels, commercial glass jars and disposable containers were used for *in vitro* culture of banana.

All cultures were incubated at 25 ± 1°C in darkness for the initiation step. After 30 days, explants were transferred to fresh medium and cultured in a 16/8-h light/dark photoperiod using cool-white fluorescent lights (50 µmoL m^{-2} s^{-1}).

Multiplication of shoot cultures

The explants were sub-cultured five times, and after each sub-culture, the mean multiplication rate was calculated as the number of shoots and buds per initial shoot. The length and fresh weight of shoots as well as the number of shoots suitable for rooting were also recorded.

Data analysis

Analysis of variance was conducted for a factorial design by the GLM procedure of SAS (SAS Institute, 2005). The initial analysis indicated significant subculture interactions; therefore, each subculture was analyzed separately. The means were separated by the Duncan test at the 5% level within each factor (culture vessel, medium, and interaction) separately in each of the subculture treatments. Means over subcultures were also compared by the Duncan test at the 5% level. The mean tables were constructed using the TABULATE procedure of SAS (SAS Institute, 2005).

RESULTS

Disinfection of the explant is the first most important step in establishing any plant in tissue

culture. By using the disinfestation method described above, 90% of the explants were successfully established. This was accomplished by adding a 10-min 0.05% (w/v) mercuric chloride treatment.

Shoot weight

The averages for shoot weight were similar among the subcultures; 2.4, 2.0, 2.6, and 1.8 g for subcultures 1, 2, 3 and 4, respectively (Table 1). The use of different culture vessels resulted in significant differences in shoot weight for subcultures 1 and 4. In subculture 1, the shoot weights were 2.8, 1.3 and 3.1 g for disposable containers, Magenta vessels, and glass jars, respectively. Similar results were obtained for subcultures 2 and 4; however, the differences were not statistically significant in these cases. Unlike these subcultures, the highest mean was obtained for jars (2.2 g), which was statistically higher than either of the other treatments (disposable = 1.7 g and Magenta = 1.6 g).

Table 1. The effects of culture vessels and medium types on the shoot weight (g) of the Dwarf Cavendish banana (*Musa acuminata* L.A. Colla) during tissue culture.

Source	Subculture				Mean
	1	2	3	4	
Culture vessel					
Disposable	2.8^{a}	2.6	2.7	1.7^{b}	2.3
Jar	1.3^{b}	1.3	2.3	2.2^{a}	1.9
Magenta	3.1^{a}	2.0	2.5	1.6^{b}	2.1
Medium					
Agar	2.3^{b}	1.7^{b}	2.1	1.3^{c}	1.7^{B}
Agargel	2.3^{b}	2.0^{ab}	2.6	1.9^{ab}	2.2^{AB}
Phytagel	2.9^{a}	2.5^{a}	2.3	2.2^{a}	2.4^{A}
Plant agar	2.0^{b}	1.8^{b}	3.3	1.7^{b}	2.2^{AB}
Interaction					
Disposable/Agar	2.6^{bcd}	2.7^{abc}	2.8	1.4^{cde}	2.0^{AB}
Disposable/Agargel	2.7^{bcd}	1.9^{bcde}	2.5	1.7^{bcde}	2.2^{AB}
Disposable/Phytagel	3.2^{bc}	3.5^{a}	2.9	1.7^{bcde}	2.9^{A}
Disposable/Plant agar	2.7^{bcd}	2.5^{abcd}	2.8	1.8^{bcd}	2.1^{AB}
Jar/Agar	1.8^{ef}	1.9^{bcde}	2.1	1.7^{bcde}	1.9^{AB}
Jar/Agargel	1.1^{f}	0.9^{e}	1.9	1.7^{bcde}	1.5^{B}
Jar/Phytagel	1.1^{f}	1.0^{e}	2.4	3.1^{a}	2.2^{AB}
Jar/Plant agar	1.2^{f}	1.6^{cde}	2.7	2.1^{b}	2.0^{AB}
Magenta/Agar	2.4^{cde}	1.1^{e}	1.4	1.0^{e}	1.3^{B}
Magenta/Agargel	3.3^{b}	3.5^{a}	3.8	2.3^{b}	3.0^{A}
Magenta/Phytagel	4.1^{a}	2.9^{ab}	1.8	1.9^{bcd}	2.2^{AB}
Magenta/Plant agar	2.3^{de}	1.3^{de}	4.1	1.2^{de}	2.3^{AB}
Mean	2.4	2.0	2.6	1.8	2.1

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ according to the Duncan test. The tests were conducted separately within each group (culture vessel, medium, and interaction). Capital letters indicate overall mean comparisons by the Duncan test at the 5% level.

The medium had no significant effect on the shoot weight during subculture 3; however, it had a significant effect in subcultures 1, 2 and 4 (Table 1). Phytagel resulted in the highest shoot weight (overall mean = 2.4 g), while agar, Agargel and plant agar resulted in 1.7, 2.2 and 2.2 g, respectively. Overall, the trends were similar among the subcultures. The interactions yielded significant differences for culture vessel and medium interactions for all

subcultures except subculture 3 (Table 1). Overall, disposable/Phytagel and Magenta/Agargel combinations yielded the highest shoot weights. The differences between the interaction means did not follow an apparent pattern. For example, in subculture 1, the Magenta/Phytagel combination yielded the greatest shoot weight (4.1 g). For subculture 2, the highest means were obtained with disposable/Phytagel and Magenta/Agargel combinations (3.5 g). Finally, the highest mean for subculture 4 was in the jar/Phytagel combination.

Shoot length

The shoot length averages increased progressively with subculture; the averages were 4.9, 5.2, 6.1, and 6.5 cm for subcultures 1, 2, 3 and 4, respectively (Table 2). The culture vessels resulted in significantly different shoot lengths for only subculture 3, where disposable and Magenta treatments had higher means than jar (6.4 *vs* 4.8 cm). Overall means were separated into

Table 2. The effects of culture vessels and medium types on the shoot length (cm) of the Dwarf Cavendish banana (*Musa acuminata* L.A. Colla) during tissue culture.

Source	Subculture				Mean
	1	2	3	4	
Culture vessel					
Disposable	5.0	5.7	6.4^{a}	6.0	6.0^{B}
Jar	3.3	4.1	4.8^{b}	5.6	4.8^{C}
Magenta	6.5	5.6	6.4^{a}	7.5	6.8^{A}
Medium					
Agar	4.9^{ab}	5.5^{ab}	6.2	6.1^{b}	5.9^{AB}
Agargel	4.6^{ab}	4.7^{ab}	6.8	7.4^{a}	6.3^{A}
Phytagel	5.8^{a}	6.0^{a}	6.0	7.1^{a}	6.4^{A}
Plant agar	4.3^{b}	4.4^{b}	5.4	6.1^{b}	5.5^{B}
Interaction					
Disposable/Agar	5.3^{abc}	5.9	6.3^{b}	5.5	5.7^{CDEF}
Disposable/Agargel	4.3^{bcd}	4.7	6.5^{b}	7.2	6.1^{BCDE}
Disposable/Phytagel	6.4^{ab}	7.6	7.4^{b}	7.1	7.2^{B}
Disposable/Plant agar	3.9^{cd}	4.9	5.1^{b}	5.8	5.4^{DEFG}
Jar/Agar	4.3^{bcd}	5.5	4.9^{b}	5.6	5.3^{EFG}
Jar/Agargel	3.3^{cd}	3.4	4.8^{b}	5.4	4.5^{FG}
Jar/Phytagel	3.1^{cd}	3.9	4.7^{b}	5.9	4.8^{FG}
Jar/Plant agar	2.5^{d}	3.3	4.9^{b}	5.5	4.7^{G}
Magenta/Agar	5.1^{abc}	5.3	6.9^{b}	7.1	6.5^{BCD}
Magenta/Agargel	6.5^{ab}	6.5	10.2^{a}	9.0	8.5^{A}
Magenta/Phytagel	7.4^{a}	6.4	5.5^{b}	7.5	6.8^{BC}
Magenta/Plant agar	7.0^{a}	4.6	5.9^{b}	7.3	6.4^{BCDE}
Mean	4.9	5.2	6.1	6.5	6.0

Different letters represent significance differences by the Duncan test at the 5% level. The tests were conducted within each group (culture vessel, medium and interaction) separately. Capital letters indicate overall mean comparisons by the Duncan test at 5%.

three groups, where Magenta was followed by disposable and jar treatments (6.8, 6.0 and 4.8 cm, respectively). Unlike culture vessel treatment, the medium type did not affect shoot length for subculture 3 but rather for all other subcultures (Table 2). Overall, the highest averages were observed with Phytagel and Agargel treatments (6.4 and 6.3 cm, respectively). These treatments had the highest means in all subcultures. The interactions were significantly different for subcul-

tures 1 and 3. Overall, the highest mean was obtained with the Magenta/Agargel combination (8.5 cm), which was among the groups with the highest means for subcultures 1 and 3.

Shoot number

The shoot number averages increased through subculture 3 (1.6, 1.9 and 2.3 for subcultures 1, 2 and 3, respectively) and then decreased to 1.9 in subculture 4 (Table 3). Culture vessel effects on shoot number were significant only for subculture 3. The overall averages for culture vessels were respectively 2.3, 1.9 and 1.8 for jar, disposable and Magenta treatments, where jar was significantly different from the others. Shoot number was significantly affected by medium only for subculture 4. Overall, Phytagel and plant agar had higher means compared to agar and Agargel (2.1, 2.1 and 1.7 and 1.9, respectively). The culture vessel × medium interaction did not result in significant differences for subcultures 1, 2, and 4 but did for subculture 3. Overall, means for combinations were among the highest mean groups and the same was true for subculture 3.

Table 3. The effects of culture vessels and medium types on the shoot number of the Dwarf Cavendish banana (*Musa acuminata* L.A. Colla) during tissue culture.

Source	Subculture				Mean
	1	2	3	4	
Culture vessel					
Disposable	1.7	2.0	2.3^{ab}	1.7	1.9^{B}
Jar	1.4	1.4	2.8^{a}	2.5	2.2^{A}
Magenta	1.7	2.0	1.9^{b}	1.6	1.8^{B}
Medium					
Agar	1.4	1.8	1.9	1.7^{b}	1.7^{B}
Agargel	1.5	1.6	2.3	1.7^{b}	1.9^{AB}
Phytagel	1.6	2.0	2.2	2.2^{a}	2.1^{A}
Plant agar	1.9	2.0	2.6	1.9^{ab}	2.1^{A}
Interaction					
Disposable/Agar	1.0	2.2	2.3^{abc}	1.8	1.9^{ABC}
Disposable/Agargel	2.0	1.8	2.2^{abc}	1.4	1.8^{ABC}
Disposable/Phytagel	1.5	2.0	1.7^{c}	1.3	1.7^{BC}
Disposable/Plant agar	2.4	2.2	3.1^{ab}	1.9	2.2^{AB}
Jar/Agar	1.2	2.0	2.2^{abc}	2.1	2.0^{ABC}
Jar/Agargel	1.4	1.2	3.3^{a}	2.3	2.2^{AB}
Jar/Phytagel	1.6	1.1	2.6^{abc}	3.3	2.4^{A}
Jar/Plant agar	1.2	1.2	3.5^{a}	2.4	2.3^{A}
Magenta/Agar	1.8	1.5	1.5^{c}	1.2	1.4^{C}
Magenta/Agargel	1.0	1.5	1.8^{bc}	1.8	1.6^{BC}
Magenta/Phytagel	1.8	2.5	2.5^{abc}	1.9	2.2^{AB}
Magenta/Plant agar	2.0	2.1	1.6^{c}	1.4	1.6^{BC}
Mean	1.6	1.9	2.3	1.9	2.0

Different letters represent significance differences by the Duncan test at the 5% level. The tests were conducted within each group (culture vessel, medium and interaction) separately. Capital letters indicate overall mean comparisons by the Duncan test at the 5% level.

DISCUSSION

We tested the effect of different culture vessels (industrial glass jars, Magenta boxes, and disposable containers) and gelling agents (agar-agar, Agargel, Phytagel, and plant agar) on

the micropropagation of Dwarf Cavendish banana to identify the combination that produces a high number of good-quality banana seedlings. When shoot number (which is the most important variable in multiplication) was considered, commercial glass jar/Phytagel and jar/plant agar combination yielded significantly higher numbers of shoots than did any other combination. The quality of the seedlings produced by these combinations, indicated by shoot weight and length in our study, was comparable to that of other combinations for shoot weight but lower than that of many other combinations for shoot length.

In a similar study, Neštáková et al. (2000) compared the effect of two gelling agents, agar and Phytagel, on *in vitro* growth and shoot development of miniature rose and gladiolus. They collected data for number of shoots, shoot length, number of leaves per shoot, and fresh weight. For both of the species studied, significantly higher fresh weight was recorded on Phytagel. Shoot multiplication was significantly higher on Phytagel for miniature rose culture, although for gladiolus, agar showed higher numbers. Conversely, the number of leaves per shoot was significantly higher on agar medium for miniature rose, and the length of the main shoot of gladiolus was higher on Phytagel. When agar and Phytagel were compared pair-wise in our results, the comparisons were either not significant or Phytagel had higher numbers than with agar. The differences in the two sets of results can be explained by the study of different species.

The cost of the disposable containers is higher than glass jars and Magenta vessels since they are used only once, which may appear to increase the long-term cost of producing banana seedlings by micropropagation. However, glass jars and Magenta vessels must be cleaned, which can add considerable cost. Therefore, depending on the labor cost necessary to clean the vessels, the overall cost of disposables may not be significantly different than for others. Plant agar is a less expensive alternative to conventional agar, which also helps to reduce overall costs. However, because we found differential patterns among vessel types and media as well as for their interactions, we suggest that selection be made based on economical factors in banana micropropagation.

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