COMPARISON OF GELLING SUBSTANCES USED IN MICROPROPAGATION

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Abstract. Seven gelling substances were evaluated for the micropropagation of *Gerbera jamesonii* 'Pink Quill' and *Hemerocallis* 'Aztec Gold'. Although multiplication and rooting for both plants were similar on media gelled with several of the gelling substances, the commercial grade of Phytagar was generally superior and Nutrient Agar inferior to the others. Normal plantlets were produced on all gels except Gelrite, the use of which resulted in watersoaked and strap-shaped leaves in gerbera cultures.

INTRODUCTION

In most cases the nutrient media used for micropropagation are gelled. Gelling is brought about by the addition of agar to the nutrient solution during preparation. Until recently, the standard agar used in research and commercial laboratories, especially in the U.S., has been Difco-Bacto agar. In the last few years, in commercial labs in particular, Bacto-agar has often been replaced by other gelling substances. This switch has been a reflection of price and improved clarity of the prepared gel.

Comparative growth of various plant species on media gelled with different agars has been the subject of several studies (1,2,6,7). The influence of agar concentration on growth of cultures has also been evaluated (2,4,5,6,7). With the explosion in the commercial use of micropropagation, it was felt that a more thorough examination of several of the commonly available gelling substances was in order.

MATERIALS AND METHODS

Gel evaluation. Seven gelling substances were tested (Table 1). In order to obtain standard curves of firmness based on gel concentration, an Instron was selected for use.

Using Murashige and Skoog salts (3) and 3% sucrose, six concentrations of each gel were prepared. The pH was adjusted to 5.7. The solutions were heated to boiling on a hot plate and 140 ml aliquots were poured into triplicate 20 × 150 mm petri dishes. After the gels had set, the dishes were covered and placed in a refrigerator overnight at 5°C. Evaluations were done the following day.

Gels were allowed to warm to room temperature prior to testing. A probe with a cross sectional area of 1.3 cm^2 was used

Table 1. Gelling substances evaluated in study.

Gel	Source	G/liter
Bacto-agar	Difco	7.0
Gelrite	Kelco	1.6
Sigma Agar	Sigma	4.6
Phytagar-I	Gibco	4.9
Phytagar-CG	Gibco	4.3
TC Agar	Gibco	5.4
Nutrient Agar	Difco	11.5

with the Instron. Near the middle of each sample the probe was inserted into the gel at a speed of 20 mm/min to a depth of 10 mm by which time the gel invariably broke. The instrument reading at the point of breakage gave an objective measure of gel firmness or strength. Curves for each gel resulting from this evaluation are presented in Figure 1. Bacto-agar at 7 g/liter was used as the standard for comparison in this study. Bacto agar at 7 g/liter had a firmness or gel strength value of 233 g/liter. The horizontal line in Figure 1 intersects the curves of the other gels at gel strengths equivalent to 7 g/liter Bacto-agar at 7 g/liter had a firmness or gel strength value of 233 g/liter. The horizontal line in Figure 1 intersects the

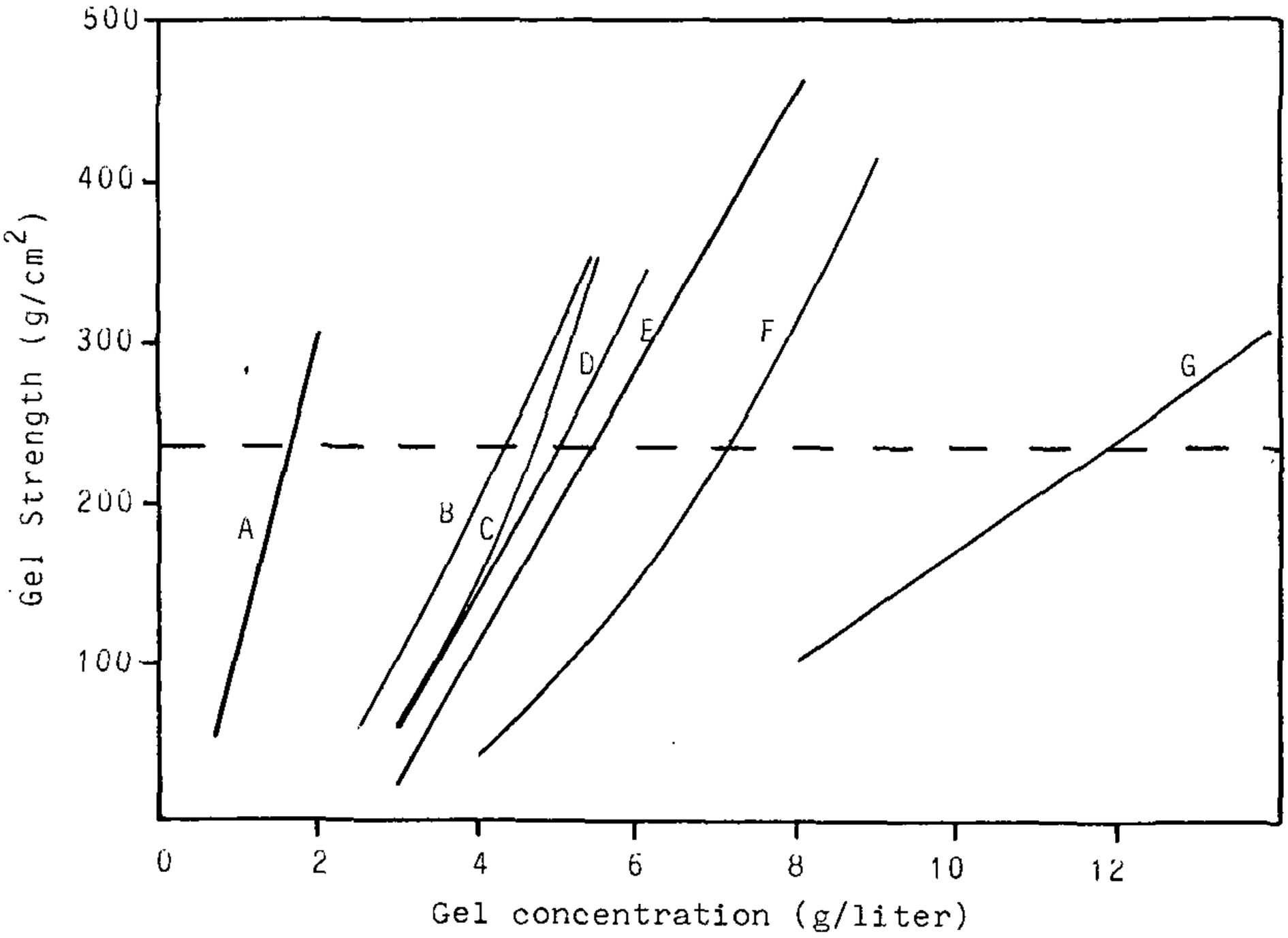

Figure 1. Standard gel strength curves for Gelrite (A), Phytagar-CG (B), Sigma Agar (C), Phytagar-I (D), TC Agar (E), Bacto-agar (F) and Nutrient Agar (G). Dashed line is at 233 g/cm^2.

Multiplication and rooting of *Gerbera*. Multiplying cultures of gerbera 'Pink Quill' were obtained from a commercial

micropropagation laboratory for the purposes of this study. A modified Murashige and Skoog medium with(in mg/l): kinetin(5) and IAA(0.5) was used for multiplication and IAA(10) for rooting. A sucrose concentration 4.5% and a pH of 5.7 was used. Three plantlets, each approximately 10 mm in height for multiplication and 20 mm in height for rooting studies were explanted into small baby food jars containing 25 ml of medium. There were 10 jars for a total of 30 explants per gel. Cultures were evaluated for multiplication after 6 weeks and for rooting after 3 weeks.

Multiplication and rooting of *Hemerocallis.* Multiplying 'Aztec Gold' cultures, obtained from the previously mentioned source were used. A modified Murashige and Skoog medium with(in mg/l): 2iP(16) was used for multiplication and IAA(10) and NAA(2) for rooting studies. A sucrose concentration of 3% and a pH of 5.7 was used.

Three plantlets, approximately 10 mm in height, were explanted into small baby food jars containing 25 ml of medium. There were 10 jars for a total of 30 explants per gel. Cultures were evaluated for multiplication after 6 weeks and for rooting after 4 weeks.

RESULTS AND DISCUSSION

Multiplication and rooting of *Gerbera.* Gerbera explants cultured in media gelled with the commercial grade of Phytagar (Phytagar-CG) formed more plantlets in general, especially when compared to those in Gelrite and Sigma Agar (Table 2). Along with the purified grade of Phytagar (Phytagar-1) and Nutrient Agar, plantlets growing in Phytagar-CG were taller. Increased height would facilitate easier handling of plantlets during subculture.

Table 2. Multiplication of *Gerbera* 'Pink Quill'[1].

Gel	Average	
	No. plantlets/culture	Plantlet ht.(mm)
Bacto-agar	7.7 ab[2]	25 c
Gelrite	6.2 b	27 bc
Sigma Agar	6.6 b	26 bc
Phytagar-I	7.9 ab	29 abc
Phytagar-CG	9.3 a	32 a
TC Agar	7.5 ab	25 c
Nutrient Agar	8.4 ab	31 ab

[1]30 explants per treatment
[2]Mean separation within columns by Duncan's multiple range test, 5% level.

The number of roots formed on the plantlets and root weight were similar with the exception of cultures grown on

Gelrite and Nutrient Agar gelled media (Table 3). In addition, root weight of plantlets on media gelled with Bacto agar was lower.

With the exception of media gelled with Gelrite, gerbera plantlets appeared normal. However, when Gelrite gel was used, the leaves were watersoaked and strap-shaped. This was true in both the multiplication and rooting stages. These plantlets were considered unsatisfactory for further use.

Table 3. Rooting of *Gerbera* 'Pink Quill'[1].

Gel	Per plantlet	
	No. roots	Root wt.(mg)
Bacto-agar	12.7 bc[2]	242 c
Gelrite	9.2 cd	286 bc
Sigma Agar	17.1 a	323 ab
Phytagar-I	17.0 ab	322 ab
Phytagar-CG	16.5 ab	360 a
TC Agar	16.5 ab	363 a
Nutrient Agar	8.9 cd	140 d

[1]30 explants per treatment
[2]Mean separation within columns by Duncan's multiple range test, 5% level.

Multiplication and rooting of *Hemerocallis*. Plantlet formation from 'Aztec Gold' cultures was similar for all gelling substances except Nutrient Agar which was inhibitory in this regard (Table 4).

Table 4. Multiplication of *Hemerocallis* 'Aztec Gold'[1].

Gel	Average no. plantlets (10 mm +)/culture
Bacto-agar	8.5 ab[2]
Gelrite	9.2 ab
Sigma Agar	9.4 ab
Phytagar-I	8.5 ab
Phytagar-CG	9.5 ab
TC Agar	7.8 b
Nutrient Agar	3.7 c

[1]30 explants per treatment
[2]Mean separation within columns by Duncan's multiple range test, 5% level.

Use of Nutrient Agar also resulted in the formation of fewer roots on subcultured plantlets and a lower root weight compared to the other gels (Table 5). In general, root weight of *Hemerocallis* plantlets on media gelled with Phytagar-CG was better.

In contrast to gerbera, *Hemerocallis* explants growing on the Gelrite gelled medium were normal in every respect.

Table 5. Rooting of *Hemerocallis* 'Aztec Gold'[1].

Gel	Per plantlet	
	No. roots	Root wt.(mg)
Bacto-Agar	10.9 a[2]	127 bcd
Gelrite	8.0 a	104 dc
Sigma Agar	9.4 a	139 bc
Phytagar-I	10.4 a	141 bc
Phytagar-CG	10.1 a	165 ab
TC Agar	8.6 a	107 cd
Nutrient Agar	4.4 b	83 d

[1]30 explants per treatment

[2]Mean separation within columns by Duncan's multiple range test, 5% level.

It is evident from the data presented that the micropropagator should examine several gelling substances before deciding on one to use as the standard for a particular plant species. In addition, cost of the gel should be considered in that the least expensive gel may be the logical choice, especially if multiplication and rooting are equivalent to the others. A comparison of prices of the various gels will show substantial differences in the cost to produce a multiplied or rooted plantlet.

LITERATURE CITED

1. Cooke R.C. 1977. The use of an agar substitute in the initial growth of Boston ferns in vitro. *HortScience* 12:339.
2. Debergh, P.C. 1983. Effects of agar brand and concentration on the tissue culture medium. *Physiol. Plant.* 59:270-276.
3. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.
4. Romberger, J.A. and C.A. Tabor. 1971. The *Picea abies* shoot apical meristem on culture. I. Agar and autoclaving effects. *Amer. Jour. Bot.* 58:131-140.
5. Singha, S. 1982. Influence of agar concentration on *in vitro* shoot proliferation of *Malus* sp. 'Almey' and *Pyrus communis* 'Seckel'. *Jour. Amer. Soc. Hort. Sci.* 107:657-660.
6. Singha, S. 1984. Influence of two commercial agars on *in vitro* shoot proliferation of 'Almey' crabapple and 'Seckel' pear. *HortScience* 19:227-228.
7. Singha, S. Townsend, E.C. and G.H. Oberly. 1985. Mineral nutrient status of crabapple and pear shoots cultured *in vitro* on varying concentrations of three commercial agar. *Jour. Amer. Soc. Hort. Sci.* 110:407-411.

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