

Alternative Gelling Agents for Potato Tissue Culture Applications

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Summary

Some cheap gelling agents were investigated as alternatives of Bacto agar. Potato (*Solanum tuberosum* L.) cv. Desiree, meristems were cultured on some agar alternatives namely: potato starch, gelatin, corn starch, (potato starch and gelatin) and ice cream thickener (carboxy methyl cellulose).

Mixing of potato starch with gelatin at concentrations of (40 and 80)g/l added to Murashiege and Skoog (1962) (MS) nutrient medium similar to that obtained when using Bacto agar (8 g/l). Survival rate, shoot height and mean shoot number were 92%, 5.2 cm and 5 shoots consequently when cultured meristem tips grew on this mixture of potato starch and gelatin. Ice cream thickener (24 g/l) was also considered fair medium for this purpose achieving 88 %, 5.2 cm and 5.1, respectively. Besides sustaining the cultures. This agar alternative was found cheap and available.

Keywords: Gelling agents; Potato micropropagation.

Introduction

As a gelling agent, Bacto agar is widely used for preparing solid and semi-solid plant tissue culture media (Scholten & Pierik, 1998). However, establishment of plant micropropagation laboratories must be based on cost effectiveness. In this respect, Bacto agar represents one of the most expensive used media components. Gelatin at a concentration of 100 g/l was tested, but is currently little used as it melts at 25 °C compared to agar which melts at 100 °C. Adaoha and Roscoe (1982) obtained normal plantlets on media solidified with methocel (methyl cellulose) and alginate as gelling agents intended for protoplast culture. However, two disadvantages appeared when using these alginates. The first is that solutions can not be autoclaved without unacceptable alginate breakdown despite that sterilization was conducted at 90 °C for 20 mins. The second disadvantage is that, alginate reacts with divalent metal cations which may cause nutrient deprivation.

Other researchers tried a mixture of agar and other compounds. Naik and Sarkar (2001) stated that sage starch could be used as a substitute for agar in culture media. They examined cassava starch, barley starch and sage. The latest was used at a concentration of 80 g/l forming a firm gelling surface throughout the entire culture period and fostered optimum plantlet growth in terms of shoot height, number of nodes per plant, number of leaves and fresh mass.

Zapata (2001) successfully reduced the cost of banana tissue culture up to 90% by replacing the tissue culture sucrose grade source with a commercial sugar. He mixed corn and potato starch with gelrite as an alternative gelling agent and the obtained results were very convincing.

Tapioca and sage were also used by Gebre and Sathyanarayana (2001) at 9%-18% and 10%-14% (w/v), respectively. Tapioca at 14%-15% stimulated growth giving significantly higher root fresh weight gaining 36.3% over agar.

The highest biomass conversion (53.3 %) was found with sage.

Puchoa, *et al* (1999) found that gelrite solidified medium produced 67.1 shoot/culture compared with 68.0 for Difco Bacto-agar. Smkolov and Lipavsk (2001) tested a mixture of starch and gellan gum (gelrite) as a low cost solidifying agent in the micropropagation of hop plants. They reduced the cost up to 50 % without exhibition of vitrification. In the literature vitrification is termed, translucency, hyperhydration, hyperhydric transformation, glauciness, waterlogging, glassiness. Vitrification is especially common if the plant has too much water available, this being usually the case in liquid media, or if the medium has a low agar concentration (Pierik, 1987).

Companies dealing with chemicals have already invested in extensive research work concentrating on potato starch as a possible candidate for Bacto agar replacement (Anonymous, 2003).

The aim of this work was to investigate the locally available cheap agar alternatives. So that the new gelling substitute may replace Bacto agar in plant tissue culture work.

Materials and Methods

Potato cultures were initiated as previously described by Naik and Sarkar (2001). Desiree cultivar was used in this investigation since it is widely planted in Iraq and well established in our lab.

Cuttings with single node were cultured on MS medium (Murashige & Skoog, 1962) and kept in a growth chamber with a photoperiod of 16 hours light and 8 hours dark at 25 ± 1 °C. Gelling agents were bought from local market and mixed with double distilled water to obtain the required concentration. Agar substitutes used in this work were, a- Carboxy methyl cellulose (which is called locally, Thalabia or Ice cream thickener) at concentrations of 8, 16, 24, 32 and 40 g/l. b- A mixture of potato starch and gelatin in a ratio of 1:2. These concentrations were 8:16, 16:32, 24:48, 32:64 and 40:80 g/l respectively. c- A mixture of potato starch and corn starch in a ratio of 1:1. Each

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was used at concentrations of 25, 37 and 50 g/l. These substances were all dissolved in a double distilled water and treated as the common procedure of Bacto agar. Preliminary experiments showed that concentrations of Bacto agar alternatives higher than the values stated above gave very solid media and were therefore excluded.

Completely randomized design (CRD) with 15 replicates per treatment was used in these experiments which were repeated twice. Least significant differences (LSD 0.05) were calculated according to Gomez and Gomez (1984).

Results and Discussion

In the preliminary experiment (Table 1), neither potato starch nor corn starch individually sustained cultures in all used concentrations. Liquid cultures were only obtained at low concentrations (200 g/l). Semi-solid media were obtained at 300 and 400 g/l, which makes the use of such agar alternatives not practical. Thalabia, appeared to be optimistic particularly at a concentration of 24 g/l since 88 % of cultured shoots survived on such solidifying agent and continued growth till a height up to 5.2 cm at the end of the third week in culture. Mean shoot number during the culture period was good giving 5.1 shoots/

Table 1. Effect of some agar alternatives on micropropagation of potatoes

Gelling agents	Conc. g/l	% of meristem survival	Mean shoot height (cm)	Mean shoot no.	Gelling status
Potato starch	200	None	---	---	Liquid
	300	None	---	---	Semisolid
	400	None	---	---	Semisolid
Corn starch	200	None	---	---	Liquid
	300	None	---	---	Semisolid
	400	None	---	---	Semisolid
Ice cream thickener	12	None	---	---	Liquid
	16	25	3.4	4.2	Semisolid
	24	88	5.2	5.1	Semisolid
Potato starch and gelatin	10 and 40	None	---	---	Liquid
	20 and 60	88	4.6	4.3	Semisolid
	40 and 80	92	5.2	5.0	Semisolid
LSD 0.05			0.3948	0.7364	

culture, respectively.

The concentration of ice cream thickener (16 g/l) resulted in 25%, 3.4 cm and 4.2 for survival, mean shoot height and average number of shoots. Mixing potato starch with gelatin, at a ratio of 1:2, increased the meristem survival up to 92%. Mixing 10 g/l potato starch and 40 g/l gelatin resulted in a liquid media. Increasing the ratio up to 20 and 60 g/l led to a semi-solid medium. Solidification only occurred with a mixture of potato starch and gelatin respectively at 40 and 80 g/l. The later improved plantlet survival up to 92% achieving the highest plantlet height (5.2 cm) with a mean shoot number of 5 shoots/culture vessel. Growth of potato single node segments on

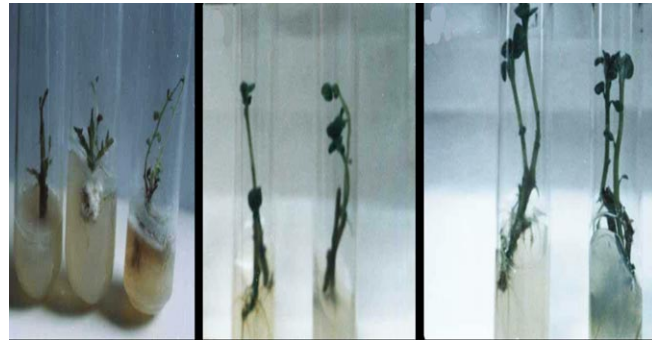


Fig. 1. Growth of Potato single node segments on MS medium free of hormones containing a mixture of gelling agents (40g/1 potato starch and 80g/gelatin) from left to right, after (12, 16 and 21 days) from culture.

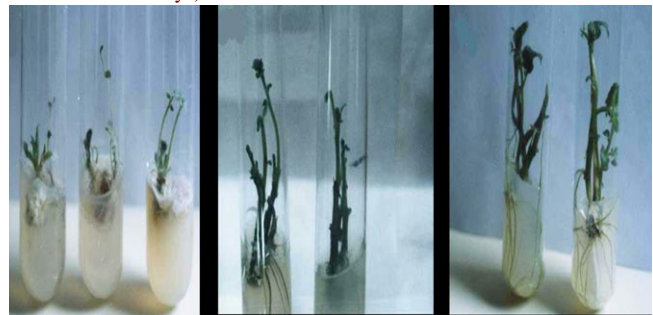


Fig. 2. Growth of potato single node segments on MS medium free of hormones solidified with ice cream thickener at a concentration (40g/1 potato starch and 80g/gelatin) from left to right, after (12, 16 and 21 days) from culture.

a mixture of potato starch and gelatin. (Figure 1) indicates the ability of the mixture to sustain shoot growth over time. Figure (2). also shows potato plantlet production on MS medium free of hormones and solidified with ice cream thickener. Plantlets were ready for transfer to the microtuberization medium at this age (3 weeks) or for taking single node cuttings for further multiplications.

In light of the results of Table (1), the two agar substitutes that proved to be suitable for potato micropropagation were compared with Bacto agar after the addition of Indole acetic acid (IAA) and Benzyl adenine (BA) (Table 2). Ice cream thickener did not significantly differ with agar in mean plant height, mean number of shoots and survival percentage at concentration of 1.5 mg/l IAA and 2.5 mg/l BA.. When IAA and BA levels were increased respectively to 2.5 and 3.5 mg/l, a slight increase in mean shoot height occurred in shoots grown on ice cream thickener and survival percentage decreased compared with Bacto agar although neither the increase nor the reduction were significantly different.

The mixture of potato starch (4%) and gelatin (8%) showed a decline in mean shoot height (5.2 cm), in mean shoot number (4.5) and in survival rate (88%) at 1.5 mg/l of IAA and 2.5 mg/l of BA compared with Bacto agar at the same auxin and cytokinin concentrations.

At the second combination of IAA (2.5 mg/l) and BA (3.5 mg/l), the mean shoot height, the shoot number and the survival rate improved and reached values approaching the Bacto agar ones.

Table 2. Performance of in vitro potatoes in some gelling agents at four levels of IAA or BA

Gelling agents	Concentration%	IAA Conc. mg/l	BA Conc. mg/l	Mean shoots height (cm)	Mean No. of shoots	Survival %
Bacto agar	0.8	0	0	4.8	3.3	88
		1.0	2.0	4.7	3.7	89
		1.5	2.5	5.1	4.6	92
		2.5	3.5	4.9	5.2	94
Corn starch	2.4	0	0	5.0	3.2	87
		1.0	2.0	5.0	3.2	86
		1.5	2.5	5.0	3.8	89
		2.5	3.5	5.2	4.5	88
Ice cream thickener Potato starch and gelatin	(4 and 8)	0	0	4.5	3.2	86
		1.0	2.0	4.7	4.0	85
		1.5	2.5	5.2	4.5	88
		2.5	3.5	4.6	5.0	95
LSD 0.05				0.3948	0.7364	7.32

It is clear that searching for new gelling agents has become crucial for plant tissue culture laboratories, particularly those involved in large scale commercial plant micropropagation. The exclusion of agar or agar alternatives from culture media may lead to hyperhydricity, a physiological disorder common to liquid culture which makes the addition of gelling agents indispensable in some cases. On the other hand, gelling agents may be a major source of unknown variation and a rich source of impurities (Puchooa, *et al.*, 1999).

This work proved the possibility of using ice cream thickener or a mixture of potato starch and gelatin (1:2) instead of Bafco agar in potatoes micropropagation projects. Low prices of these gelling agents encourage such use.

The current prices in the Iraqi local market are US\$2/kg for ice cream thickener and one US\$/kg for potato starch compared with US\$100/kg for Bacto agar (Jan. 2005). Al-

though agar substitutes may differ in their composition than agar, which may affect culture growth, this phenomenon was not observed in potato cultures. Investigating the use of such agar alternatives on other plant species is crucial.

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بدائل لمادة الأجار في تطبيقات أكثر البطاطس بزراعة الأنسجة النباتية

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الخلاصة

تم اختبار بعض بدائل الأجار رخيصة الثمن في الإكثار الدقيق للبطاطس (*Solanum tuberosum* L.) صنف ديزيره Desiree. زُرعت المرستيمات القمية على هذه البدائل التي شملت نشا البطاطس، الجيلاتين (الهلام)، نشا الذرة الصفراء، خليط نشا البطاطس مع الجيلاتين إضافة إلى مثخن الأيس كريم والذي هو عبارة عن مادة كاربوكسي ميثيل السليلوز والذي يسمى محلياً بالثعلبية.

أوضحت نتائج الدراسة بأن خليط نشا البطاطس مع الجيلاتين وبتراكيز 40 و80 غم/ لتر على التوالي والمضافة الى الوسط الزراعي MS أنتجت وسطاً صلباً مشابهاً إلى الوسط المصلب بإضافة باكتو آجار (8 غم/ لتر). تم حساب نسبة البقاء، ارتفاع الأفرع وعدد الأفرع الناتجة وكانت 92%، 5.2 سم و5 فرع على التوالي بعد زراعة المرستيمات القمية على خليط نشا البطاطس مع الجيلاتين. كما أن مثخن الأيس كريم وبمعدل 24 غم/ لتر اعتبر وسطاً مناسباً لهذا الغرض محققاً 88%، 5.2 سم و1.5 من نسبة البقاء وطول الأفرع وعددها على التوالي، علماً بأنه رخيص الثمن ومُتوفر محلياً.

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