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COST REDUCTION IN TISSUE CULTURE: II - CHEAPER SUBSTITUTES FOR AGAR

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ABSTRACT

When using cheaper substrates instead of agar, perlite gave the significant heaviest *Gerbera* shoots, the highest number of shoots and the greatest significant leaf number. *Gerbera* shoots grown on perlite had the highest content of total chlorophyll.

In case of *Lilium*, the significant heaviest shoots and the highest number of shoots were a result of using cotton as a substrate. The effect of substrate type on shoot length was significant on *Lilium*. Longest *Lilium* shoots were those grown on peat moss. Greatest significant leaf number and the highest content of total chlorophyll were detected in shoots grown on cotton.

Keywords: agar, substrate, cotton, peat moss, perlite, sand.

INTRODUCTION

This study aims to prove that it is possible to use cheaper substitutes instead of the expensive agar in the multiplication stage of two plants belonging to different classes,i.e.Dicotyledonae (*Gerbera jamesonii*) and Monocotyledonae (*Lilium longiflorum*). Proving this will help to:

1- Reduce the monthly costs of the tissue culture technique by using a cheaper substrate in place of agar.

2 - Saving a big deal of hard currency paid to import agar from abroad.

MATERIALS AND METHODS

This work was carried out in the Tissue Culture Laboratory, Zohriya Garden, Agricultural Research Center,Cairo,during the period from July to December 2004.

In *vitro*-produced explants of *Lilium longiflorum* (basal parts of the leaves) and *Gerbera jamesonii* cv. Festival (shoots) were used as a starting material.

Glass jars of 11.5 cm height x 6.5 cm dia. with their polypropylene caps were used.

For each plant,50 ml of the liquid Murashige and Skoog (1962) (MS) medium were poured in four groups of these jars. Certain amounts of cotton, peat moss, perlite or washed sand, as substrates, were put in each of these groups as shown in Table (a). Medium in a fifth group of jars was solidified with agar at 6 g/l. All media were supplemented with benzyl adenine (BA) at 5 ppm for the *Gerbera* explants or 3 ppm for the *Lilium* ones. These 5 groups of jars represented treatments that were arranged in a completely randomized design. Each treatment comprised 12 replicates (jars).

Explants were inoculated on the media under aseptic conditions using a laminar airflow cabinet. Jars were incubated for four weeks at 25/20°C (day/night) ±2°C, 70% relative humidity.Two fluorescent tubes/shelf were installed at 30 cm above explants to provide light intensity of 2200-2400 lux at explant level for 16 h. daily.

Table (a): Size and weight of a substrate/jar

Substrate	Size (ml)	Weight (g)
Cotton	50	3
Peat moss	50	15
Perlite	50	5
Sand	50	100

Data obtained in the multiplication stage were: shoot fresh weight (g), shoot number, shoot length (cm), leaf number and shoot content of total chlorophyll (mg/g fresh weight). Data were statistically analyzed using SAS 1995 computer program, and means were compared by L. S. D. method according to Snedecor and Cochran (1980).

Analysis of some characteristics of these substrates was conducted in the labs of the Horticultural Institute. Results of this analysis are shown in Table (b).

Table (b): Analysis of the used substrates

	pH	Total dissolved salts (ppm)
Distilled water	5.60	4
Cotton	5.60	4
Peat moss	4.50	30
Perlite	6.25	65
Sand (before washing)	8.50	500
Sand (after washing)	6.0	10

A feasibility study was conducted to assess the costs of using these types of substrates.

RESULTS

Effect of substrate type at multiplication stage (Tables 1 and 2):

1- Effect of substrate type on shoot fresh weight:

The effect of substrate type on *Gerbera* shoot fresh weight was found to be significant. Heaviest shoots were those grown on perlite, followed with a significant difference by those grown on agar medium. Shoots grown on cotton, peat moss and sand were not significantly different and were the lightest.

In case of *Lilium*, the corresponding effect was also statistically significant.However,the heaviest shoots were those grown on cotton, followed, without any significant difference by those grown on peat moss. Shoots grown on perlite were significantly lighter than those grown on cotton, but insignificantly different from shoots produced on either peat moss from

one side, or sand and agar from the other side. Shoots of the later two substrates were the lightest at all.

2- Effect of substrate type on shoot number:

Shoot number was significantly influenced by substrate type in both *Gerbera* and *Lilium*. Number of *Gerbera* shoots was the highest when perlite was used. The second category in a descending order, comprised number of shoots grown on either cotton or agar medium, without a significant difference in between. Lowest significant shoot number resulted from using either peat moss or sand as substrates.

In case of *Lilium*, the significant highest number of shoots was a result of using cotton as a substrate, followed with a significant difference by peat moss. Number of shoots grown on agar medim, although lower, was not significantly different from those grown on peat moss from one side or on perlite from the other side. The significant lowest shoot number was a result of using sand, though not significantly different from number of shoots grown on perlite.

3- Effect of substrate type on shoot length (cm):

The effect of substrate type on *Gerbera* shoot length was insignificant, though it was significant on *Lilium* shoot length. Longest *Lilium* shoots were those grown on peat moss, followed without any significant difference by those grown on cotton. Lilium shoots grown on perlite, sand or agar were significantly the shortest, though they were not significantly shorter than those grown on cotton.

Table 1 - Effect of substrate type on multiplication of *Gerbera jamesonii*

Water quality	Shoot fresh weight (g)	Shoot No.	Shoot length (cm)	Leaf No.	Total chlorophyll (mg/g fresh weight)
Cotton	0.27 c	11.33 b	2.33 a	18.67 c	1.65
Peat moss	0.22 c	6.33 c	2.43 a	16.67 cd	1.45
Perlite	1.02 a	16.33 a	2.27 a	42.00 a	1.95
Sand	0.17 c	7.67 c	2.60 a	12.67 d	1.35
Agar	0.68 b	11.00 b	2.07 a	36.33 b	1.77
L.S.D. at 5%	0.20	2.24	N.S.	4.36	

Table 2 Effect of substrate type on multiplication of Lilium

Water Quality	Shoot fresh Weight (g)	Shoot No.	Shoot Length (cm)	Leaf No.	Total Chlorophyll (mg/g fresh weight)
Cotton	1.83 a	9.33 a	10.45 ab	15.00 a	1.72
Peat moss	1.59 ab	3.33 b	13.40 a	5.67 bc	1.45
Perlite	1.06 bc	1.67 cd	9.33 b	3.67 cd	1.18
Sand	0.80 c	1.00 d	7.37 b	1.33 d	1.36
Agar	0.99 c	2.57 bc	8.80 b	7.12 b	0.87
L.S.D. at 5%	0.55	1.61	3.26	2.51	

4- Effect of substrate type on leaf number:

The greatest *Gerbera* leaf number belonged to shoots grown on perlite, followed with a significant difference by those grown on agar. Leaf number of *Gerbera* shoots grown on cotton was significantly lower than that of shoots grown on agar, though insignificantly different from that of shoots grown on peat moss. The later, in turn was insignificantly different from leaf number of shoots grown on sand, which scored the lowest significant record.

In case of *Lilium*, greatest significant leaf number was observed on shoots grown on cotton, followed with a significant difference by those grown on agar. Number of leaves produced on perlite was not significantly different from those produced on peat moss from one side, and from those grown on sand from the other side. The later substrate produced the significant least number.

5- Effect of substrate type on total chlorophyll content (mg/g fresh weight):

Gerbera shoots grown on perlite had the highest content of total chlorophyll, followed in a descending order, by those grown on agar, cotton, peat moss and sand. The later was lowest at all.

In case of *Lilium*, the highest content of total chlorophyll was detected in shoots grown on cotton, followed in the same order, by those grown on peat moss, sand, perlite and finally agar, which gave the lowest content.

DISCUSSION

Among the many problems facing the tissue culture technique is the big sum of foreign currency needed to purchase the expensive agar from abroad. Workers over a long period of time have tried a lot of cheaper substitutes for agar especially when some problems of depending on agar were noticed.

Adelberg *et al* (1992) stated that mericlones of *Brassolaeliocattleya* and a *Laeliocattleya* hybrid were cultured on modified MS medium which was either solidified with agar or covered by A microporous polypropylene membrane (Celgard) floats. Proliferation on the membrane resulted in more harvestable plants than with agar medium.

Barbas *et al* (1993) reported that agar inhibited growth, induced mature leaf formation and necroses of the *in vitro* cultured shoots of hybrid walnut (*Juglans regia* X *Juglans nigra*). Agar altered the chemical composition of the medium as well as that of the explants. A pronounced accumulation of Na and several microelements was observed in leaves after 16 days of culture on agar, probably due to a disturbance in the K selectivity mechanism and membrane permeability. Mineral element accumulation was evident after growth inhibition. Lack of growth, mature foliar morphology, Na and microelement accumulation support the hypothesis that agar accelerates the ageing of *in vitro* propagated walnut trees. Nairn *et al* (1995) stated that tissue cultured *Pinus radiata* grown on a medium containing agar as the gelling agent display toxic symptoms and poor long-term shoot survival. A constituent could be separated from low molecular weight components of the agar responsible for the toxic symptoms by dialysis. It was identified as being

an agaroid-type xylogalactan bearing pyruvate and sulfate substituents. Nand *et al* (1995) showed that optimal rooting and plantlet growth of sugarcane cv. B.O.91 on MS basal medium with 0.5 mg BA and 0.5 mg NAA/litre was observed at 0.75% (w/v) agar level. Root and shoot proliferation was inhibited by high agar concentrations.

Agar was praised by some authors such as Punia *et al* (2000) when they studied the effects of using liquid media with support matrices (nylon cloth, muslin cloth, glass wool or filter paper) on sugarcane (cultivars CoH92 and CoH99) shoot multiplication. They found that shoot multiplication decreased sharply when liquid media containing nylon and muslin cloth, glasswool, or filter paper were used.

However, Saadawy (2000) stated that for the multiplication of the two orchid *Laelia anceps* and *Cymbidium devonianum* explants, corn starch could be used to some extent instead of the expensive agar. Gebre and Sathyanarayana (2001) conducted a study to compare the efficacy of cheap alternative gelling agents, tapioca (from tubers of *Manihot esculenta*) and sago (from the stem pith of *Metroxylon sagu*), and agar for *in vitro* shoot regeneration and microtuber production using nodal explants of potato. They reported that the type of gelling agent significantly affected *in vitro* plant regeneration of nodal explants of potato. After 32 days of incubation, shoot height in agar reached 8.9 cm with 10.5 nodes. Shoot height ranged between 8.9 and 9.8 cm in tapioca (at the concentration of 11-15%) with 11.3-12.1 nodes. Sago-based medium was generally unfavourable for *in vitro* shoot proliferation. Tapioca at 14-15% stimulated growth giving significantly higher root fresh weight gain (36.3%) over the agar. The effect of gelling agent was more pronounced on microtuber fresh weight than on microtuber number. A differential increase in weight occurred in the media solidified with either tapioca or sago. Within a concentration of 11-14% of tapioca, the existence of a favourable osmotic environment and better carbohydrate and ionic supplement is suspected, which may have improved cell growth and morphogenesis. The results showed the possibility of a successful use of tapioca for *in vitro* culture of potato. El-Kazzaz and El-Bahr (2003) found that the best medium for developing the date palm cv. Samany plantlets *in vitro* was a liquid half MS medium with perlite as substrate, which gave the highest number of roots, root and shoot length. Sansberro *et al* (2003) ascertained that rooting of regenerated shoots of *Schinopsis balansae* (Anacardiaceae) was observed on 1/4 MS medium with vermiculite as the substrate and supplemented with 7.5 μM (1.5 ppm) IBA.

Feasibility study

In addition to the expenses of the different salts, vitamins, amino acids and growth regulators needed to prepare the different types of tissue culture media; agar represents another expensive and indispensable component of solid media. To do without agar, i.e. using liquid media means the necessity to use an electric shaker, which in turn is also expensive (about L.E. 5000).

The use of another material to act as a substrate might be useful in cutting down the high cost of tissue culture technique. It will also mean that about L.E. 1500 needed to buy a magnetic stirrer (necessary to prepare the

agar-solidified media) can be saved. Prices and costs of the different substrates studied as agar substitutes in this paper are shown in Table (c).

Using cotton as a substrate means a big deal of savings in the tissue culture process. Other materials also make a good reduction in the overall costs of tissue culture labs. The problem with sand is that it does not absorb liquids as the other substrates. So, an over layer of media solution could be seen that might represent a handicap of explant respiration. Further studies need to be done in order to overcome this obstacle in order to make use of the fabulous cheap price of this easily available material.

Table (c): Prices of the different substrates

Substrate	Price of 1 kg (in L.E.)	Weight of substrate needed for 100 liter (kg)	Total costs for 100 liter of medium (L.E.)
Cotton	0.500	6	3
Peat moss	2.000	30	60
Perlite	10.000	10	100
Sand	0.004	200	0.8
Agar	250.000	0.600	150

CONCLUSION

Significant heaviest *Gerbera* shoots were those grown on perlite. Shoots grown on cotton, peat moss and sand were the lightest. The significantly heaviest *Lilium* shoots were those grown on cotton. Shoots of sand and agar were the lightest at all.

Shoot number was significantly influenced by substrate type in both *Gerbera* and *Lilium*. Number of *Gerbera* shoots was the highest when perlite was used. Lowest significant shoot number resulted from using either peat moss or sand as substrates.

In case of *Lilium*, the significantly highest number of shoots was a result of using cotton as a substrate. The significantly lowest shoot number was a result of using sand.

The effect of substrate type on *Gerbera* shoot length was insignificant, though it was significant on *Lilium* shoot length. Longest *Lilium* shoots were those grown on peat moss. Lilium shoots grown on perlite, sand or agar were significantly the shortest.

The greatest significant *Gerbera* leaf number belonged to shoots grown on perlite. Sand, which scored the lowest significant record. In case of *Lilium*, greatest significant leaf number was observed on shoots grown on cotton. Sand produced the significant least number.

Gerbera shoots grown on perlite had the highest content of total chlorophyll. Sand gave the lowest at all. In case of *Lilium*, the highest content of total chlorophyll was detected in shoots grown on cotton. Agar resulted in the lowest.

Recommendations

It is recommended to search for cheaper substitutes for the expensive agar to be used as a substrate or matrix of the tissue culture media. Cotton

and perlite gave considerably better results compared to agar in many explant characteristics during the multiplication stage. Further studies should be carried out in order to make use of cheaper substrates such as sand.

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تقليل تكاليف زراعة الأنسجة: ٢ – بدائل أرخص للآجار

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عند إستعمال مواد أرخص ثمنا بديلا للآجار ، فإن البيرلايت أنتج أثقل أفرع الجربيرا وأكثرها عددا وأكبر عدد من الأوراق . وبلغ محتوى أوراق الجربيرا من الكلوروفيل الكلى أعلاه عند إستعمال هذه المادة . وفى حالة الليليوم فإن أثقل الأفرع وأكثرها عددا نتجت عند إستعمال القطن مهدا للمنفصلات . وكان تأثير نوع المهد على طول الفرع معنويا ، حيث كانت أطول الأفرع هى تلك النامية على البيت موس . ونتج أكبر عدد من الأوراق وأعلى محتوى من الكلوروفيل الكلى فى الأفرع النامية على القطن .