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Short communication

Low Cost Particle Matrices: Alternatives for Agar in *In vitro* Sub-Culturing of *Anthurium andreanum*

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ABSTRACT. *Agar used in in vitro cultures of plants is relatively expensive. This study attempt to replace it from locally available, low-cost and eco-friendly inert particles as demonstrated in Anthurium in vitro sub-culturing. A laboratory experiment was conducted with the particle matrices in liquid media. Four particle types were used (sand, coir dust, charcoal and tile pieces; 1-2 mm) at different volume ratios (1:1:1:2, 1:1:2:1, 1:1:3:1, 1:1:1:1, 1:1:1:3, 0.5:1:1:1, 0:0:1:0, 0:0:0:1). It was found that all types of the inert particles produced better plants than the agar control, with added advantages of lower microbial contamination, higher rates of acclimatization and growth of plantlets and hence immense economic gain with cut off of the sub-culturing steps. Mixed particle ratio (1:1:2:1) was the best matrix for in vitro sub-culturing of Anthurium. The use of the best particle matrix increased the profit margin by 10 -fold compared to agar.*

INTRODUCTION

Micro propagation is currently applied to a large number of floricultural species, including *Anthurium*. However, it is costly due to intensive manipulations throughout the various culture phases. Agar, which is relatively expensive, has been used in *in vitro* cultures of plants for more than a century. In recent studies, inert materials were tested for replacing agar in the *in vitro* cultures. Luffa sponge, coir and inert particles were observed to enhance rooting of various plant species compared to agar (Gangopadhyay *et al*., 2002; Gangopadhyay *et al.,* 2004; Seneviratne *et al*. 2004). Therefore, the present study was conducted with the aim of replacing agar from inert particle matrices in liquid media, using *Anthurium* as the test plant.

MATERIALS AND METHODS

Four particle types (sand, coir dust, charcoal and tile pieces; 1-2 mm) at different volume ratios (1:1:1:2 (T_1), 1:1:2:1 (T_2), 1:1:3:1 (T_3), 1:1:1:1 (T_4), 1:1:1:3 (T_5), 0.5:1:1:1 (T_6),

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0:0:1:0 (T_7), 0:0:0:1 (T_8)) were used. Fifty grams of the each mixture were added into the glass bottles separately. Then, Murushige and Skoog (MS) liquid medium (30 ml) was added to them as the plant nutrient medium. MS medium supplement with 0.75% plant agar (Duchefa Biochemie, The Netherlands) was used as the control (T_9). Eight months after regeneration, *Anthurium* plantlets with initial roots (1-2 roots; 1 mm long, four leaves) in the MS liquid medium supplemented with 1 mg/l BAP and 2 mg/l 2-4 D were transplanted in the autoclaved particle media and the control. Then plants were incubated under 16 h photoperiod at a light intensity of 40 – 80 µmol $m^{-2}s^{-1}$ and at 25 ^oC with a 78% relative humidity. The control was sub-cultured four times during five months of the study period, due to deterioration of the medium, whereas the particle media were kept without sub-culturing. Each treatment was replicated 15 times and experiment was arranged in a Completely Randomized Design (CRD). Five months after transplanting, growth parameters of plants (leaf number, fresh weight, root number, length and fresh weight of 2 cm root base, shoot height, plant fresh weight and plant dry weight, contamination and survival rates when acclimatization) were recorded. The CEC of the particle matrices were determined by using atomic absorption spectrophotometer. Data were analyzed using GLM procedure of the SAS (1996) software, and means were separated using Tukey's HSD test. Benefit/cost ratio was estimated for comparison of the best particle matrix and the agar control.

RESULTS AND DISCUSSION

Treatments 6 (0.5:1:1:1) and 8 (0:0:0:1) had high CEC, 32 and 27 cmol/kg, respectively compared to other treatments. Media with higher CEC help retain nutrients.

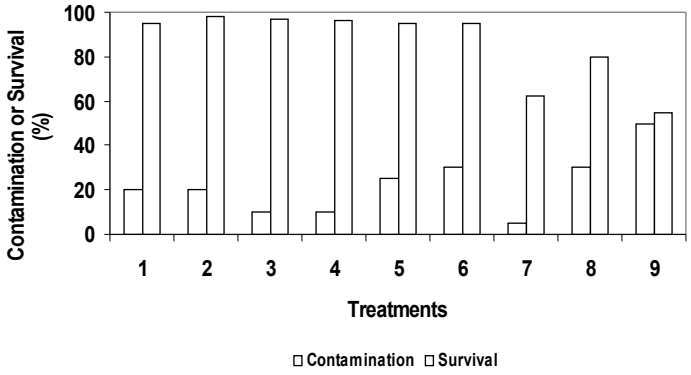

Figure 1. Contamination or survival of *in vitro* sub-cultured *Anthurium* of particle matrices used for different treatments, as of table 1 (n = 100)

Plant dry weight was highest in the mixture of 1:1:2:1 (Table 1). In all particle cultures, plant height increased compared to the control. However, the ability of retaining nutrients of the particle matrices, as revealed by the CEC was not reflected in the plant growth. Higher plant survival rates were observed in all particle matrices (Figure 1). In addition, a higher microbial contamination and lower survival rates were observed in the agar control (Figure 1). Table 2 shows the estimate of benefit/cost ratio of the best particle matrix (1:1:2:1) compared to agar, for the production of 36,000 plants. It clearly shows that the use of the

particle matrix is extremely profitable compared to conventional agar cultures for sub-culturing. The use of the particle matrix increases the profit margin by 10-fold compared to agar. As such, mixed particle (1:1:2:1) matrix was the best medium for *in vitro Anthurium* propagation. More importantly, these particle cultures when used can save a large sum of money spent on agar and cut down the sub-culturing steps for saving labour, particularly because the particles can be re-used after sterilization. In conclusion, after root initiation, the particle matrices are superior to agar, because the same matrix can be used through *in vitro* sub-culturing to acclimatization of *Anthurium*. Further, this technique should also be tested for other plants.

Table1. Growth parameters of *in vitro* sub-cultured Anthurium with different ratios of the inert particle matrices and the agar control

Treatment (particle ratio)	Plant height (cm)	Plant fresh weight (g)	Plant dry weight (g)	Leaf number/ plant	Root number/plant
T_1 (1:1:1:2)	4.90ab	0.073b	0.007b	3.80a	4.40ab
T_2 (1:1:2:1)	3.75bc	0.191a	0.018a	4.60a	4.40ab
T_3 (1:1:3:1)	4.17bc	0.059b	0.007b	3.10a	2.90abc
T_4 (1:1:1:1)	4.66abc	0.107ab	0.010ab	3.80a	3.00abc
T_5 (1:1:1:3)	6.1a	0.153ab	0.012ab	4.60a	4.80a
T_6(0.5:1:1:1)	3.75bc	0.118ab	0.009b	3.40a	3.30abc
T_7 (0:0:1:0)	4.02bc	0.071b	0.007b	3.00a	2.10c
T_8 (0:0:0:1)	3.75bc	0.075b	0.009b	3.10a	2.40c
T_9 (control)	3.30c	0.115ab	0.012ab	4.20a	2.70bc
MSD (0.05)	1.50	0.095	0.009	1.97	1.93
CV (%)	24.7	62.0	64.5	36.8	40.4

Table 2. Estimate of benefit/cost ratio of the best particle matrix (1:1:2:1) compared to agar of *in vitro* sub-cultured *Anthurium*. This is for 36,000 plants, which can be produced by using 1 kg of agar

Item	Agar	Particle matrix
Cost (Rs.)		
- Raw materials	77,073.00	390.00
- Labour		
- media preparation	3,600.00	18,000.00
- sub-culturing	21,600.00	14,400.00
- cleaning jars	6,000.00	2,000.00
Total	108,273.00	34,790.00
Benefit (Rs.)		
- Income	36,00,000.00	36,00,000.00
- Contamination	-14,40,000.00	-720,000.00
- Mortality	-12,96,000.00	-28,800.00
Total	864,000.00	28,51,200.00
Benefit/cost ratio	**8.0**	**82**

REFERENCES

Gangopadhyay, G., Bandyopadhyay, T., Gangopadhyay, S.B. and Mukherjee, K.K. (2004). *Luffa* sponge a unique matrix for tissue culture of *Philodendron*. Curr. Sci. 86: 315-319.

Gangopadhyay, G., Das, S., Mitra, S.K., Poddar, R., Modak, B.K. and Mukherjee, K.K. (2002). Enhanced rate of multiplication and rooting through the use of coir in aseptic liquid culture media. Plant Cell Tiss. Org. Culture. 68: 301-310.

SAS (1996). The SAS for windows, Ver. 6.12. Statistical Analysis Systems Institute Inc., Cary, NC.

Seneviratne, K.A.C.N., Wijesundara, D.S.A., Dhanasekera, D.M.U.B., Vitharana, G.S.P.K. and Palipane, P.W.U.B. (2004). Potential of replacing agar with inert particles for *in vitro* culture of Orchids (*Dendrobium* spp). ASDA 6: 325.