Study on the Low-Cost Methods for Tissue Culture Applications in Orchid

P.U. Kumara, A.G.K.M.W.S. Atapattu*, F. M. M. T. Marikar

General Sir John Kotelawala Defence University, Ratmalana, Sri Lanka

* arsc@kdu.ac.lk

Abstract - Orchid is one of the most important cut flowers, grown in Sri Lanka. Quality raw material supply is a key success for orchid cultivation and scarcity leads to major concern in sustainable growth of the industry in a long run. Mass cultivation by micro propagation techniques can be successfully used to mass propagation of orchids; thus, cost benefit is less due to the high cost. Aim of this research was to replacement of expensive agar with low-cost substance for *in vitro* sub culturing of Cattalya seedlings. Two-month-old Cattleya seedlings were subcultures on Murashige and Skoog media (MS) as basal media. As gelling agents' sago, semolina, corn flour, semolina + agar, corn flour + agar was used. Used agar as the control treatment. Cultures were maintained inside the culture room. Number of leaves, Number of roots, Leaf length, leaf width and percentage of contaminations were recorded at four-week intervals. Sago and semolina + agar showed higher performance and low contamination percentage as alternative gelling agent in the MS media. The results of this study propose that sago, semolina, and corn flour can be used as an alternative gelling agent in MS media. This low-cost method might be uplift the orchid culture in Sri Lanka.

Keywords: Alternative gelling agent, Alternative water source, Orchid subculture

I. INTRODUCTION

Orchid is very popular and widely used as a decorative garden plant, cut flower and as a potted plant. It is most important and popular cut flower in the world. It has different colors and color combinations which attract the beauty of the flower as unique. Since orchid flower has a long life as an ornamental flower leads to high market share in cut flower industry.

Commercial grown orchid varieties are produced by cross pollination, and they are multiplied by micro propagation for commercial market. The development of in vitro method has vastly improved the germination of orchid seeds and accelerates the production of orchid plants. Introduction of the tissue culture technology is useful to produce high quality planting material all over the world [1]. Recently the orchid industry is expanding with tissue culture technology.

Agar is the most widely used gelling agent for the preparation of solid and semi-solid media [2]. This contributes to matrix potential, humidity, and affects the availability of water and dissolved substances within the culture vessel. Research results have shown that sago, semolina, corn, wheat, laundry starch, rice and potato flour can be used as agar substitutes [3, 4]. This study is focused on, to evaluate the effect of alternative media components and development of Cattleya seedlings in Sri Lankan context.

II. MATERIALS AND METHODS

MS media was prepared as basal media. Control experiments were performed on 7 g/l agar using different concentrations of different gelling agents to solidify the medium. Distilled water was used as the water source. pH was adjusted into the range of 5.6-5.80. Solution was heated by using gas cooker. When the nutrient solution was boiling, gelling agent was dissolved by thoroughly stirring. The media was poured into sterilized empty jam bottles covered it by cellophane film. 45ml of media was poured into each bottle, and media height was about 1.5cm. Finally, the media was autoclaved under 121°C and 1.5kg/cm^2 for 20 minutes.

Table 1. Different gelling agents and their weight for treatments in MS media

Treatment	Agar (g)	Sago (g)	Semolina (g)	Corn flour (g)
T1	7			
T2		120		
T3			90	
T4				90
T5	3.5		45	
T6	3.5			45

Mature old leaves of two-month-old *in vitro* cultured seedlings were cut and removed. using sterilized tools under laminar flow cabinet seedlings were established on different gelling agent media bottles by using forceps. Four seedlings were planted in one jam bottle. The culture bottles were sealed well with cellophane films and maintained in an air-conditioned growth room at 26 ± 2 °C, 25 % RH and under fluorescent illumination with 16-hour photoperiod. After about 4 weeks (at this point plants were well established in the medium) the established plants were transferred to a same media after recording the plant growth parameters. They were also maintained under the same conditions as described.

Each treatment was consisting of 10 replicates and there were four seedlings per one experimental unit. All the seedlings were uprooted carefully from the culture media and kept on the petri plate. Then numbers of root, number of leaves were counted. Leaf length and leaf width were measured using 1mm graph paper attached on the bottom of petri plate.

Data were recorded at intervals of 4 weeks to 3 months. Experiments were organized as a fully randomized design with 10 replicates. Data were analyzed using the SAS software package and significant differences between treatments were identified using his ANOVA procedure at the 5% probability level. Mean separation was performed using Duncan's multi-interval test.

III. RESULTS AND DISCUSSION

When using sago as a gelling agent no significant difference was not observed in growth parameters compared with control (agar). The contamination occurred in sago medium was 13%. Semolina treated medium was not significantly affected on the growth parameters, except leaf length when comparing with control. Leaf length showed the lowest significant difference compared with agar medium. Also, the contamination percentage is quite higher than in other medium. It was 20% contamination occurred in semolina medium.

Corn flour treated medium was not significantly affected on growth parameters except number of leaf & leaf length, when compared with agar medium. Number of leaf and leaf length showed lowest significant difference compared with agar medium. The contamination percentage was 12% in corn flour medium. Semolina and agar medium was not significantly affected on all growth parameters, when compared with control. The contamination percentage was 13 %. Corn flour and agar mixture of gelling agent also not significantly affected on growth parameters except for number of leaves compared with control. Number of leaves showed lowest significant difference compared with control, while making the contamination percentage is 11% in this medium.

IV. Results and Discussion

According to the results it can be concluded that agar can be replaced with Sago and Agar + Semolina as gelling agent in MS media.

References

[1] S. Prasad, U. Kumar, and D. Singh, “Commercial Floriculture,” Agrobios publication, Cambridge, 1998.
[2] J. Arditti, and R. Ernst, “Micropropagation of orchids,” John Wiley and Sons, New York, 1993.
[3] E. Nagamori, and T. Kobayashi, “Viscous additive improves micropropagation in liquid medium”. J. Biosci. Bioengin., Vol 91: Pp. 283-287, 2001.
[4] D. Sivakumar, R. S. W. Wijeratnam, F. M. M. T. Marikar, M. Abeyesekere, and R. L. C. Wijesundera. "Antagonistic effect of Trichoderma harzianum on post-harvest pathogens of rambutans.", Acta horticulturae, 2001.